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Efficacy of prostacyclin on oleic acid-induced hypoxemic respiratory failure in the dog

by

J. Hugh Devitt

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

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J. Hugh Devitt

Efficacy of prostacyclin on oleic acid-induced hypoxemic respiratory failure in the dog

Thesis presented for Master of Science 1985

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submitted by J. Hugh Devitt

in partial fulfilment of the requirements for the degree of Master of Science

Supervisor

Date June 13/85

ABSTRACT

Prostacyclin may improve hemodynamics and diminish pulmonary injury in the oleic acid model of the adult respiratory distress syndrome. This beneficial effect is believed to be due to vasodilatation and platelet aggregation inhibition. Both pharmacological actions are thought to be mediated via adenylate cyclase raising intracellular cyclic AMP concentrations. Aminophylline is a phosphodiesterase inhibitor that demonstrates a synergistic effect when combined with prostacyclin in vitro. These actions were studied in the intact dog utilizing radiolabelled platelets, a quadruple lumen pulmonary artery catheter and an aortic cannula to study platelet kinetics and hemodynamics after oleic acid-induced lung injury. The animals were divided into six groups: i - controls, ii - oleic acid injection, iii - oleic acid injection and prostacyclin infusion, iv - oleic acid injection and aminophylline infusion, v-oleic acid injection, prostacyclin and aminophylline infusions and, vi - prostacyclin infusion only. Hemodynamic parameters as well as blood sampling for platelets and indium^{111} count rate were undertaken before and after oleic acid administration. Prostacyclin and/or aminophylline were infused for four hours commencing one

hour after the oleic acid injection. The dogs were sacrificed at the end of the infusion period and the lungs removed for further examination.

Prostacyclin infusion resulted in an average reduction of MAP of 26% when compared to dogs given oleic acid. When aminophylline was added to prostacyclin a 37% reduction in MAP occurred. Prostacyclin increased cardiac output by 50% whereas prostacyclin combined with aminophylline resulted in a 37% increase in cardiac output when compared to dogs receiving oleic acid alone. When SVR was examined, prostacyclin produced a 56% reduction while the combination of aminophylline and prostacyclin resulted in a 57% reduction in SVR. The changes in cardiac output, SVR and MAP were statistically significant when prostacyclin was infused. Only the combination of prostacyclin and aminophylline infusions blunted the rise in pulmonary artery pressure induced by oleic acid. Neither prostacyclin nor aminophylline by themselves produced a significant reduction of PAP in dogs given oleic acid. No significant differences in $Pa0_2$ were seen in any of the treatment groups.

The prostacyclin groups tended to have a higher $P\bar{v}0_2$ than the non-prostacyclin groups. These differences, however, were not significant. Examination of venous and

arterial platelet counts revealed no trends. An arterial-venous gradient in indium^{111} count rate occurred, suggesting pulmonary platelet sequestration. Finally, all animals given oleic acid showed a significant increase in lung wet/dry ratios and indium uptake suggesting pulmonary platelet entrapment with lung injury. No differences in count rate or wet/dry ratios were observed in the various treatment groups.

It is concluded that prostacyclin improves tissue oxygen and substrate delivery by elevating cardiac output in this model. Prostacyclin infused in doses known to inhibit platelet aggregation, in vivo, appeared to have little effect on pulmonary injury, as measured by wet/dry ratio, light microscopy and pulmonary uptake of radiolabelled platelets. Therefore modification of platelet aggregation, in vivo, with prostacyclin does not appear to have any beneficial action in the oleic acid model of pulmonary injury.

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LIST OF ABBREVIATIONS

$A\text{-}aD0_2$	Alveolar-arterial partial pressure gradient for oxygen
$A\text{-}VD0_2$	Arterial-venous oxygen content gradient
ACD	Acid citrate dextrose
ADP	Adenosine diphosphate
ARDS	Adult respiratory distress syndrome
CAMP	Cyclic adenosine monophosphate
$Ca0_2$	Oxygen content of arterial blood
$Cc'0_2$	Oxygen content of pulmonary capillary blood
$C\bar{v}0_2$	Oxygen content of venous blood
ECHMO	Extracorporial membrane oxygenation
EDTA	Ethylenediamine tetracetate
HHT	Hydroxyheptadecalnienoic acid
HETE	Hydroxyeicosatetraenoic acid
Hgb	Hemoglobin
HPETE	Hydroperoxeicosatetraenoic acid
KeV	Kiloelectron volt
MAP	Mean arterial pressure
MDA	Malondialdehyde
NS	Normal saline
$PaC0_2$	Partial pressure of carbon dioxide in arterial blood
P_A0_2	Partial pressure of oxygen in the alveolus
$Pa0_2$	Partial pressure of oxygen in arterial blood

PAP	Pulmonary artery pressure
PG	Prostaglandin
P_I0_2	Partial pressure of oxygen in inspired air
PIP	Peak inspiratory pressure
PPP	Platelet poor plasma
PRP	Platelet rich plasma
$P\bar{v}0_2$	Partial pressure of oxygen in the mixed venous blood
PVR	Pulmonary vascular resistance
$\dot{Q}_L$	Lung lymph flow
$\dot{Q}_S/\dot{Q}_T$	Calculated venous admixture
$\dot{Q}_T$	Cardiac output
R	Respiratory quotient
$Sa0_2$	Oxygen saturation of arterial blood
SV	Stroke volume
$S\bar{v}0_2$	Oxygen saturation of venous blood
SVR	Systemic vascular resistance
Tx	Thromboxane
Tris	Tris (hydroxymethyl)aminomethane
$\dot{V}0_2$	Oxygen consumption
$\dot{V}/\dot{Q}$	Ratio of ventilation to perfusion

REVIEW OF THE LITERATURE

ARDS Pathology and Pathophysiology

The constellation of symptoms and signs characterized by severe dyspnea, decreased lung compliance and diffuse bilateral pulmonary infiltrates on chest radiography make up the adult respiratory distress syndrome (ARDS). ARDS is a syndrome or a cluster of symptoms and signs and not a disease state per se. The etiology of this entity is diverse and includes shock states, trauma, infection (systemic or pulmonary), disseminated intravascular coagulation, embolic phenomena, aspiration, inhaled toxins, pancreatitis, oxygen toxicity, drugs viz. narcotics or salicylates, head injury and radiation injury (Petty and Fowler, 1982, Hudson 1982, Balk and Bone, 1983). Fowler et al (1983) demonstrated that while age and sex have little effect on the incidence and mortality of ARDS, increasing the number of predisposing factors such as those listed above, will increase both incidence and mortality. Despite modern monitoring techniques and current supportive therapy, this syndrome still has a mortality of 50% or greater (Petty and Fowler, 1982). While a multitude of factors have been implicated in the etiology of ARDS, the response of the lung to injurious or noxious stimulae is limited. Thus the final common pathway of

pulmonary injury remains poorly understood. Apparently, once the final sequence of events has been initiated, at present little can be done to alter the outcome.

The pulmonary pathological features of ARDS were first systemically examined by the multihospital collaborative extracorporeal membrane oxygenation (ECMO) study (Pratt et al, 1979). This group found a strong correlation between the type of pathological findings and the duration of the respiratory failure. Briefly, the pathological findings in ARDS can be broken down into injury and recovery phases. Initially, acute alveolar and interstitial edema occurs. As well, electron microscopic (EM) examination suggests both endothelial and epithelial cellular injury. This is followed by sloughing of the epithelial cell layer and hyaline membrane formation, the hyaline membranes being composed of sloughed necrotic cellular material and fibrin. The endothelial cells appear to be more resistant to widespread cellular disruption and necrosis. However, severe endothelial cell injury results in the breakdown of the air-blood interface. During recovery, alveolar septal thickening with proliferation and infiltration of interstitial cells with or without interstitial or intra-alveolar fibrosis is observed. The epithelial side of the interstitial membrane becomes lined with cuboidal

cells which contain multiple lamellae. These cells are believed to be proliferatory type II alveolar cells and are felt to be the epithelial stem cell of repair. Final recovery will contain varying degrees of fibrosis (Bachofen and Weibel, 1982).

The overall result is interstitial edema and alveolar flooding due to increased permeability and alveolar collapse because of a deficiency of surface active material. While interstitial edema is likely to have little effect on gas exchange, alveolar flooding and collapse leads to lung units which are poorly ventilated but still perfused, in other words, a $\dot{V}/\dot{Q}$ ratio which approaches zero (Dantzker 1982). The resulting intrapulmonary shunt leads to subsequent arterial hypoxemia. Since cardiac output rises only minimally and hemoglobin concentration remains essentially unchanged, venous desaturation follows to allow for adequate tissue oxygenation. Because venous blood entering the lung is further desaturated, the portion shunted through the lung will lower $Pa0_2$ further when mixed with oxygenated blood leaving adequately ventilated lung units. Thus, a positive feedback cycle is set in motion in which as $P\bar{v}0_2$ falls, $Pa0_2$ falls, which in turn leads to a further reduction in tissue oxygen availability and $P\bar{v}0_2$. This cycle can be broken by increasing cardiac output,

hemoglobin concentration or by reducing the intrapulmonary shunt. However, nonselective increases in pulmonary blood flow may worsen pulmonary shunt and decrease $Pa0_2$. Carbon dioxide excretion only becomes a problem in the end stage of the disease if the pulmonary vascular bed is obliterated and this pathophysiological event will not be discussed further.

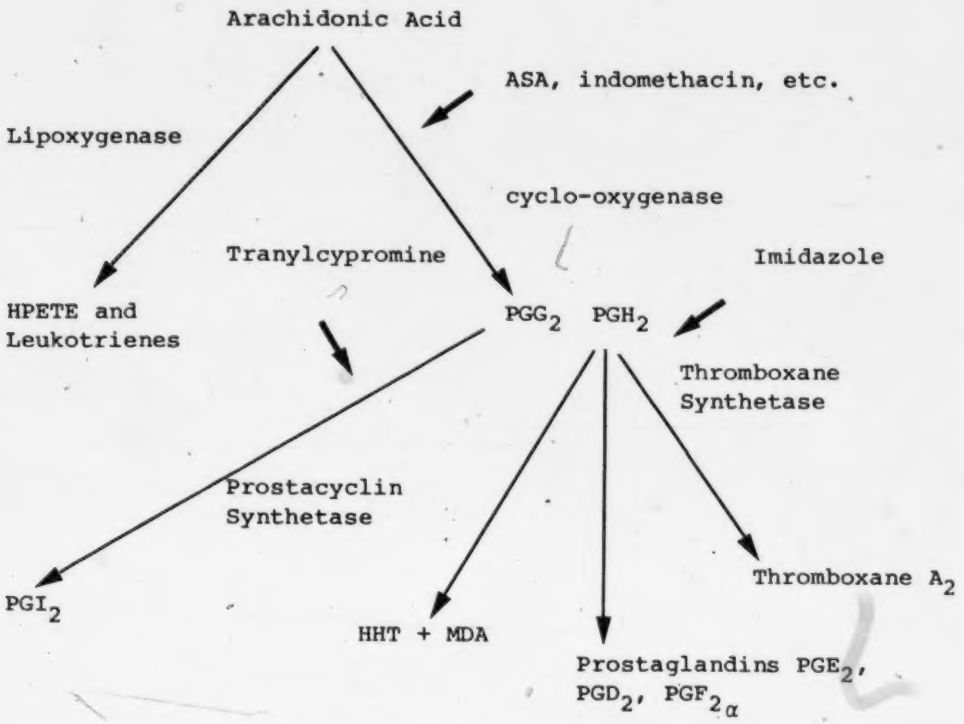
Prostaglandins - A Brief Review of Synthesis and Pharmacology

The pharmacological actions of seminal fluid, such as vasodepression and smooth muscle stimulation, were first discovered independently by Goldblatt and Von Euler in the 1930's. Compounds with these properties were named prostaglandins. These agents were purified and classified into the E and F series by Bergström in 1957. However, in the early 1970's several highly unstable compounds with potent biological activites were discovered. These included the prostaglandin endoperoxides (Hamberg et al, 1974a), thromboxane A_2 (TxA_2) (Piper and Vane, 1969, Hamberg et al, 1975) and prostacyclin (PGI_2) (Bunting et al, 1976, Moncada et al, 1976).

Arachidonic acid, a 20 carbon organic acid, is the parent compound for the leukotrienes and the bisenoic

prostaglandins. Arachidonic acid is obtained from the diet or synthetized in vivo from linoleic acid, an essential fatty acid. It readily binds to albumin and is universally incorporated into the phospholipids of cell membranes, thus providing a vast depot from which to draw. Arachidonic acid is released from phospholipids in the cell membrane by phospholipase A_2. This reaction can be enhanced by mechanical stimulation or an immunological reaction (Kunze and Voigt, 1981, Flower and Blackwell, 1976). Corticosteroids may exert their effect by inhibition of phospholipase A_2 and thereby diminish the amount of arachidonic acid available as substrate for the oxidative enzymes (Hamberg et al, 1974b). From this point, the biosythetic pathway diverges (see Fig. 1). First, arachidonic acid can enter the leukotriene system through metabolism by the enzyme lipoxygenase to produce 12-5-hydroperoxy arachidonic acid (5-HPETE) (Hamberg and Samuelsson, 1974b). This pathway will not be discussed further. Secondly, arachidonic acid may be converted to the cyclic endoperoxides, prostaglandin G_2 and H_2 (PGG_2 and PGH_2), by cyclo-oxygenase (Hamberg et al, 1974a, Hamberg et al, 1974b). Cyclo-oxygenase may be competitively inhibited by drugs such as indomethacin (Vane 1971), ibuprofen (Adams et al, 1969), or irreversibly inhibited by acetylsalicylic acid (ASA)

Figure 1 - Metabolic Pathway of Arachidonic Acid



Heavy arrows indicate points of inhibition.

(Vane 1971, Roth et al, 1974). Should these drugs be introduced, it has been postulated that more arachidonic acid would be shunted into the leukotriene pathway and an excess of lipoxygenase products would occur. Perhaps this explains the sensitivity of some patients to salicylates and similar pharmacological agents.

Oleic acid has also been shown to inhibit prostaglandin production. Using a sheep vesicular gland preparation, Pace-Asiak and Wolfe demonstrated a 55% reduction in prostaglandin production when oleic acid was also present. The mechanism for this is unclear although oleic acid may be a competitive inhibitor of prostaglandin synthesis (Pace-Asiak and Wolfe, 1968).

The cyclic endoperoxides PGG_2 and PGH_2 can undergo three independent transformations to be sythesized: 1) prostaglandins of the E and F series; 2) thromboxane A_2 (TxA_2); or, 3) prostacyclin (PGI_2) (see Fig. 1). A fourth transformation linked to thromboxane A_2 production has also been described. This results in the formation of 12-hydroxy-5, 8, 10-heptadecotrienoic acid (12-HHT) and malondialdehyde (MDA). Hamberg and Samuelsson's group have demonstrated that prostaglandin E_2 and F_2 (PGE_2 and $PGF_{2\alpha}$) are derived from the cyclic endoperoxides, PGG_2 and PGH_2 (Hamberg and Samuelsson, 1973, Hamberg et al, 1974b). The second major product of

endoperoxide metabolism is TxA_2 (Needleman et al, 1976, Hamberg et al, 1975). This reaction is mediated by the enzyme thromboxane synthetase which can be selectively blocked by imidazole (Moncada et al, 1977a). Large quantities of this enzyme are found in platelet microsomes. The third major product of endoperoxide metabolism is PGI_2. PGI_2 is produced by the enzyme prostacyclin synthetase (Moncada et al, 1976). This enzyme is found in large concentrations in vascular endothelium. Prostacyclin synthetase activity can be blocked by tranylcypromine, a competitive inhibitor (Gryglewski et al, 1976). The fourth pathway resulting in HHT and MDA production can occur enzymatically or non-enzymatically (Yoshimito et al, 1977, Hammarström and Falardeau 1977). The latter pathway is mentioned for completeness only.

Pharmacology of Prostacyclin

PGI_2, a product of the cyclo-oxygenase and endoperoxide metabolism, has a molecular weight of 374.45.

By far the greatest tissue manufacturer of this agent is the endothelial cell throughout the entire vascular tree on both arterial and venous sides of circulation. There is perhaps a slightly greater

production of prostacyclin in the arterial tree (Moncada et al, 1978). In arterioles, the greatest production of prostacyclin occurs at the intimal surface of the vessel with a gradient of decreasing production toward the adventitia (Moncada et al, 1977). MacIntyre and coworkers (1978) demonstrated that while cultures of endothelial cells manufactured prostacyclin when incubated with PGH_2 or arachidonic acid, none was produced when fibroblasts or smooth muscle cells were exposed to the same conditions. The basal rate of secretion of prostacyclin in normal man has been estimated to be within the range of 0.08-0.1ng/kg/min, using a steady state infusion technique and assessing the urinary metabolites (Fitzgerald et al, 1981). Prostacyclin is extremely unstable and possesses a half-life of 2-3 minutes at normal body temperature and pH (Dusting et al, 1978). Up to sixty percent undergoes spontaneous non-enzymatic hydrolysis to form a relatively inactive product 6-keto-$PGF_{1\alpha}$ (Rosenkrantz et al, 1981). This reaction presumably occurs in all vascular compartments. Any remaining prostacyclin is eliminated by organs such as the liver and kidneys following enzymatic degradation. The lung does not appear to metabolize PGI_2 to any great extent. The liver contains

9-hydroxyprostaglandin dehydrogenase, a nonspecific enzyme which degrades prostacyclin to 6-keto-prostaglandin E_1. While this entity has no vasoactive properties, it inhibits platelet aggregation (Quilley et al, 1980). The kidney contains 15-hydroxy-prostaglandin dehydrogenase which produces the metabolite 15-keto, 13,14-dehydro PGI_2 (Wong et al, 1979). Both these metabolites, as well as 6-keto-$PGF_{1\alpha}$, undergo further oxidation to biologically inert forms and are mainly eliminated in the urine with only a small amount undergoing fecal excretion.

Exogenously infused prostacyclin has several important pharmacological effects. First, it is perhaps the most potent inhibitor of platelet aggregation known. In this regard, other prostaglandins such as PGE_1, PGD_2 and 6-keto $PGF_{1\alpha}$ are 40, 20 and 1000 times less potent respectively than prostacyclin (Whittle et al, 1978). Their relationships closely correspond to platelet cyclic AMP concentrations when exposed to the prostaglandins mentioned above (Tateson et al, 1977). An in vitro concentration of prostacyclin in the range of 0.75-1ng/ml is all that is required to inhibit platelet aggregation induced by ADP (Wittle et al, 1978). In human studies, an infusion of 5ng/kg/min inhibited ADP induced aggregation while doses at 20ng/kg/min doubled

the template bleeding time, presumably by inhibiting the platelet phase of the coagulation cascade. Other hematological parameters such as prothrombin time, partial thromboplastin time and euglobulin lysis time remained unchanged (Gryglewski et al, 1978). Increasing the concentration of the prostacyclin infusion will continue to increase the bleeding time to a maximum of 100ng/kg/min. From this point, further increases in the amount of prostacyclin infusion had no effect on the bleeding time (Ubatuba et al, 1979).

The second important pharmacological action of prostacyclin is that of vasodilation. This occurs in all vascular beds, both arterial and venous, and is the result of direct stimulation of smooth muscle cells presumably via a specific prostacyclin receptor. The overall hemodynamic effect of a prostacyclin infusion in healthy animals is a reduction in arterial, pulmonary artery and wedge pressure as well as a fall in systemic and pulmonary vascular resistances, while in healthy subjects there is no change in cardiac output or myocardial contractility. Reflex tachycardia does occur, probably mediated through the baroreceptor reflex mechanism (Kadowitz et al, 1978, Triulzi et al, 1983, Fitzpatrick et al, 1981). As a vasodilator, prostacyclin is four times more potent than prostaglandin E_2 and 128 times more potent than 6-keto-prostaglandin $F_{1\alpha}$

(Armstrong et al, 1978).

Both the previously mentioned pharmacological actions are thought to be mediated through cyclic AMP. Two prostacyclin specific receptors have been found on platelets as well as guinea pig peripheral arterioles (MacDermott et al, 1981, Williams et al, 1983). Cyclic AMP production is stimulated by binding to the receptor (Tateson et al, 1977, Best et al, 1977). Prostacyclin is a more potent stimulator of adenylate cyclase than isoproterenol in human umbilical vein endothelial cells (Hopkins and Gorman, 1981). By selectively inhibiting cyclic AMP phosphodiesterase with theophylline, the action of prostacyclin on cyclic AMP production can be greatly augmented (Hidaka et al, 1979, Asano et al, 1977, Whittle et al, 1978).

A third pharmacological action of PGI_2 is its ability to modulate leukocyte function. Several studies have demonstrated a prostacyclin-induced inhibition of polymorphonuclear leukocytes ability to adhere to nylon fibres (McGillen et al, 1980, Boxer et al, 1980). This leukocyte adherence inhibition appears to correlate with rise in intracellular cyclic AMP. The time sequence of rise and decline of intracellular cyclic AMP levels mimicked that of adherence inhibition. Boxer and coworkers (1980) also demonstrated that polymorphonuclear

leukocytes treated with PGI_2 retained their bacteriocidal capacity. These studies suggest that by modulating polymorphonuclear leukocyte adhesion, lung damage could be minimized in those clinical entities in which pulmonary leukostasis has been implicated (Craddock et al, 1977, Jacob 1980). However, this theory has yet to be tested in an intact animal model.

The Role of Polymorphonuclear Leukocytes in ARDS

While a number of experimental models have demonstrated polymorphonuclear leukocyte sequestration in the lung leading to pulmonary dysfunction such as hypoxia and increased lung lumph flow (Craddock et al, 1977, Jacobs, 1980). Wedmore and Williams (1980) demonstrated that prior leukocyte depletion in the zymosan-activated plasma model markedly diminished edema production in the lung, but did not abolish it completely. They proposed that pulmonary edema production is multifactorial with involvement of complement, leukotrienes, leukocytes and endothelial cells (Wedmore and Williams, 1980). While methylprednisolone diminished aggregation of zymosan-activated polymorphonuclear leukocytes in vitro, prostaglandin inhibitors such as salicylates had little effect (Hammerschmidt et al, 1979). These studies suggested leukocyte behaviour in inflammation is governed

by the leukotriene system. The role of prostacyclin in leukocyte function in vivo remains to be determined, but the in vitro data suggests a modulatory action.

The Role of Platelets in ARDS

The role of platelets in the induction and propagation of ARDS remains controversial. While collagen infusions have been proposed as a model of platelet-induced pulmonary injury, changes in pulmonary vascular resistance and physiological shunt produced by this mechanism were transient and disappeared after the termination of the collagen infusion (Vaage 1982). Secondly, no interstitial or alveolar edema could be demonstrated at necropsy in this model. Therefore, this method of inducing pulmonary injury is probably not a valid model for the study of ARDS.

Pulmonary platelet sequestration certainly occurs in lung injury, induced by either oleic acid or endotoxin (Hechtman et al, 1978a). However, these trapped platelets do not appear to cross the capillary-alveolar membrane, as pulmonary lavage fluid from oleic acid injured lungs in rabbits given indium^{111} radiolabelled platelets demonstrated minimal indium activity (Spragg et al, 1982). Animals rendered thrombocytopenic by antiplatelet serum prior to lung injury induced by

pseudomonas bacteria demonstrated unaltered pulmonary leukocyte sequestration (Myrvold 1976). As well, Hechtman and coworkers calculated the arterial-venous platelet gradient in patients after abdominal aortic aneurysm repair or during septicemia and concluded that there was no correlation between pulmonary platelet entrapment and pulmonary function (Hechtman et al, 1978b). It would appear that while platelet sequestration does occur during pulmonary injury, this action has very little pathological or physiological significance.

The Role of Prostaglandins in ARDS

The role of prostaglandins in the generation or maintenance of ARDS is at best confusing. Most information on this topic is derived from the endotoxin induced lung injury model. When E. Coli or pseudomonas are injected as endotoxin or live bacteria into awake sheep previously instrumented to produce a chronic lung lymph fistula, a biphasic response occurs (Demling et al, 1981, Brigham et al, 1974, Ogletree and Brigham, 1982). Phase I, which occurs one hour after injection, is characterized by severe pulmonary hypertension and a moderate increase in lung lumph flow ($\dot{Q}_L$). However, the lung lymph protein concentration fell with respect to the

plasma protein concentration suggesting that the increased $\dot{Q}_L$ was solely on the basis of raised capillary hydrostatic pressure. Phase I was approximately one hour in duration. Three to five hours after endotoxin injection, the second phase took place. Phase II was characterized by only moderate pulmonary hypertension when compared to Phase I but a marked increase in $\dot{Q}_L$, with no change in lung lymph protein concentration. This indicated an increased lung protein clearance and suggested that the rise in $\dot{Q}_L$ was on the basis of increased capillary permeability. Metabolites of both prostacyclin and thromboxane A_2 have been found in increased concentrations in lung lymph fluid and serum after administration of endotoxin (Frolich et al, 1980, Butler et al, 1982). While the significance of this is uncertain, Rienes and his group (1982) correlated the serum concentration of the thromboxane A_2 metabolite, thromboxane B_2, with mortality rates in humans suffering from endotoxic shock. Cyclo-oxygenase inhibitors, such as indomethacin or meclofenamate, will abolish Phase I pulmonary hypertension and modest increase in lung lymph flow in the endotoxin pulmonary injury model. These drugs, however, have no effect on Phase II pulmonary hypertension and actually increased lung lymph flow and lung protein clearance in the second phase (Ogletree and

Brigham, 1979, 1982). In fact, Ogletree and his coworkers have found raised concentrations of 5-HETE, a product of the lipoxygenase pathway in their model of endotoxic lung injury and peak lung lymph 5-HETE concentration appears to correspond with the onset of phase II (Olgetree et al, 1981). They have theorized that cyclo-oxygenase inhibitors promoted shunting of mobilized arachidonic acid into the lipoxygenase pathway and it is these products, such as the slow reacting substance of anaphylaxis, that augment Phase II permeability (Olgetree and Brigham, 1982). Therefore while prostaglandins are the chief causes of Phase I pulmonary hypertension and elevated pulmonary vascular resistance, they play no role in the events of the second phase. The agent primarily responsible for the Phase I events appears to be thromboxane A_2. Thromboxane B_2 serum concentration correlated directly with the degree of pulmonary hypertension (Fletcher et al, 1981). The effect of thromboxane synthetase inhibitors have been studied specifically in the endotoxin pulmonary injury model. Casey and coworkers pretreated rats with OXY 1581, a selective thromboxane synthetase inhibitor. Rats treated as above prior to endotoxin administration did not develop early pulmonary hypertension. As well, plasma thromboxane B_2 concentration did not rise in the

pretreated rats (Casey et al, 1982). Thromboxane and cyclo-oxygenase inhibitors, decrease mortality in endotoxic shock models (Fletcher and Ramwell, 1977, Wise et al, 1980, Cooke et al, 1980). The source of thromboxane A_2 appears to originate from platelets sequestered in the lung during the initial phase of injury. By rendering test animals severely thrombocytopenic, with the use of antiplatelet serum prior to endotoxin administration, the initial rise in pulmonary artery pressure and pulmonary vascular resistance (Phase I) can be attenuated with little or no effect on Phase II (Bridenberg et al, 1977, 1980). It would appear that Phase I is transient in nature having little bearing on the degree of lung injury.

Infusion of exogenous prostacyclin before and during endotoxin administration has produced mixed results. Demling and his group started their prostacyclin infusion at the time of endotoxin administration and continued it several hours afterwards. A dose between 100-200 ng/kg/min was used. They found prostacyclin attenuated the rise in pulmonary artery pressure and pulmonary vascular resistance in both Phase I and II. However, there was only a mild reduction in $\dot{Q}_L$ suggesting that the permeability lesion was still present and the fall in $\dot{Q}_L$ was due solely to a reduction in the microvascular

hydrostatic pressure. They concluded that platelet sequestration and aggregation played a minimal role in the endotoxin lung injury model (Demling, 1981b). Fletcher and Ramwell (1980) using the endotoxin septic shock model, started a prostacylin infusion 15 minutes before endotoxin administration and continued it for 4 hours. They observed a marked decrease in mortality in the endotoxin plus prostacyclin group when compared to endotoxin alone. A dose of 20ng/kg/min was used and no effect on pulmonary arterial pressure was observed, although a small decrease in systemic arterial pressure took place. Flynn and Demling (1982) observed a reduction of thromboxane B_2 in lung lymph in animals infused with prostacyclin at a dose of 100ng/kg/min. Of note in most studies where prostacyclin has been demonstrated to be a beneficial agent in endotoxic shock or endotoxic pulmonary injury, the prostacyclin was started before the endotoxin administration, a highly impractical situation in the clinical setting.

Other models of pulmonary injury have been less well studied. The oleic acid model of ARDS is particularly devastating from a pathophysiological point of view, but little is known of what role prostaglandins have to play. There is a suggestion unsupported by any data, that the breakdown product of prostacyclin, 6-keto

$PGF_{1\alpha}$, is found in increased amounts in the arterial blood after oleic acid lung injury (Miyazawa et al, 1982). Pretreatment with cyclo-oxygenase inhibitors in 2 test models appears to have no effect whatsoever (Pace et al, 1980, Dickey et al, 1981). This is not surprising as the pulmonary response to oleic acid induced lung injury is monophasic with a steady rise in pulmonary artery pressure, pulmonary vascular resistance and endothelial permeability. There is not an early prostaglandin-induced rise in pulmonary artery pressure with oleic acid lung injury and all the events observed in this model appear to correspond to Phase II of endotoxin-induced pulmonary injury. A possible explanation for this is that oleic acid is a weak inhibitor of prostaglandin synthesis (Pace-Asiak and Wolfe, 1968). In other words, there are major differences between the two models and conclusions derived from one system may not be applicable to the second.

The use of prostacyclin in oleic acid-induced lung injury may offer several potential benefits. First, prostacyclin is a potent pulmonary vasodilator (Watkins et al, 1980). A second beneficial effect is that prostacyclin inhibits platelet aggregation in vitro and in vivo. The optimal infusion dose for this appears to be 100ng/kg/min (Ubatuba et al, 1979, Moncada et al,

1976). A third and final benefit which requires further study at this time is that prostacyclin may reduce the release of inflammatory mediators from leukocytes via a cyclic AMP-dependent pathway (Weksler et al, 1980).

Prostacyclin has been used in oleic acid-induced pulmonary injury with some promising preliminary results. Slotman and his group (1979), studied seven dogs after pulmonary injury with 0.075ml/kg of oleic acid. Three dogs received an infusion of prostacyclin at a dose of 300µg/kg/min commencing one hour after the oleic acid injection for an infusion duration of one hour. The remaining four dogs received a saline infusion one hour after oleic acid injection and were designated as the control group. They reported that the one hour prostacyclin infusion produced a significant reduction in mean arterial pressure and systemic vascular resistance when compared to the control group or the preoleic acid injection values. Cardiac output fell markedly in the control group while the prostacyclin infusion group tended to maintain cardiac output near the preoleic acid levels. As well, the fall in arterial partial pressure of oxygen ($Pa0_2$) was significant when compared the preoleic acid level in the control group only. There were however no significant intergroup differences in $Pa0_2$. No significant changes were seen in pulmonary

vascular resistance between groups although the pulmonary vascular resistance tended to rise with time in both groups. Finally, while the fall in the A-aDO_2 of the saline controls, when compared to the preoleic acid level, was significant there were no intergroup differences when compared to the prostacyclin group. It was concluded that prostacyclin exerted its beneficial effect in oleic acid-induced pulmonary injury by "preservation of hemodynamic and pulmonary gas exchange parameters at pre-injury levels". They also concluded that prostacyclin improved tissue oxygenation (Slotman et al, 1979). Slotman and his group, again reviewed the problem in 1980 in a study very similar to their previous one. This time, 300ng/kg/min was infused for the duration of one hour after the injection of 0.075m/kg of oleic acid into dogs. Again three dogs received the prostacyclin infusion one hour after the oleic acid administration, while four dogs received saline infusions and were designated as controls. While this study confirmed the finding of the first one, that is, prostacyclin infusion decreased mean arterial pressure and systemic and pulmonary vasuclar resistances when compared to the controls, the pulmonary histology was also studied. As with the previous study, no significant intergroup differences were found in cardiac output.

However, PGI_2 significantly increased the arterial venous oxygen content gradient when compared to the saline controls.

The lungs were examined histologically by an independent individual, although lung sectioning had been performed by the author. Obviously, sampling site is extremely important in oleic acid-injured lungs. Histology can range from normal areas to those with severe alveolar flooding and hemorrhage all within the same lung. Examination of the lung in the control group revealed typical oleic acid-induced lesions ranging from acute focal edema with mild inflammation to diffuse alveolar edema with an acute inflammatory infiltration. However, pathological examination of lungs of dogs infused with prostacyclin revealed essentially normal pulmonary architecture. This positive histological effect was attributed to pulmonary vascular dilatation and deaggregation of platelet clumps. While the alveolar-arterial gradient for oxygen was not reported, the authors concluded that tissue oxygenation was improved and pulmonary vasoconstriction secondary to hypoxia was eliminated (Slotman et al, 1982).

Miyazawa and his group (1982) infused prostacyclin methylester 200ng/kg/min thirty minutes after a 0.09ml/kg pulmonary artery injection of oleic acid. The control

group received only a comparable quantity of Ringers lactate buffer, one half hour after oleic acid administration. A significant reduction in minute ventilation, respiratory rate and physiological dead space was found in the group infused with prostacyclin. There were no significant differences in arterial PO_2. These authors concluded that prostacyclin exerted its effect by decreasing lung capillary fluid filtration, relaxing airway smooth muscle and deaggregating platelets (Miyazawa et al, 1982).

As can be seen, prostacyclin appears to have a multiplicity of potential beneficial pulmonary actions. Our study was developed to determine which of the previously mentioned effects were important by studying both platelet kinetics and lung edema accumulation. It was anticipated that it would be possible to find where prostacyclin exerts its beneficial effect.

MATERIAL AND METHODS

All experiments had approval of the Surgical Medical Research Institute Animal Handling Committee at the University of Alberta. The experimental protocol was divided into two phases: the first phase occurred on Day 1 and consisted of radiolabelling of platelets; and the second phase occurred on day 2 and comprised the hemodynamic study and infusion of prostacyclin.

Platelet Radiolabelling

The technique for radiolabelling platelets was modified from that described by Thakur et al (1976) and Scheffel et al (1977). Basically Indium is complexed to oxine, a lipophylic moiety which penetrates and binds nonspecifically to cell membranes in the absence of the plasma protein transferrin. $Indium^{111}$ has a half-life of 2.8 days and delivers two gamma photons at 173 and 247 KeV. This makes $Indium^{111}$ ideal for external scintigraphy (McIlmoyle et al, 1977, Goodwin et al, 1978).

All 36 adult mongrel dogs underwent autologous platelet labelling on the day prior to the prostacyclin trial. They were tranquillized with acepromazine maleate (1mg/kg) given intramuscularly. Thirty minutes after the injection, blood was obtained by venesection from a

forelimb vein. A total of 43ml of blood was withdrawn slowly into a 50ml syringe already containing 7ml of acid-citrate-dextrose (ACD) solution (see appendix 1a for composition). An 18 gauge needle and a 5 minute collection time was used to minimize the shear forces and prevent damage to the platelets during collection. The blood and ACD solutions were gently mixed by inversion. 12.5ml aliquots of the resulting blood ACD mixture was placed into each of four 15ml silicone coated glass Vacutainer tubes (Becton Dickinson). These tubes were then placed in a IEC model CM centrifuge and spun at 190g for 18 minutes to separate the platelet rich plasma (PRP) from the red blood cells. The PRP was gently aspirated into a syringe using an 18 gauge needle leaving approximately 1cm of plasma above the packed red cells in each vacutainer tube. The PRP was then pooled in a new 15ml silicone coated vacutainer tube and recentrifuged at 1400g for 15 minutes to pellet the platelets. The platelet poor plasma (PPP) was carefully removed and set aside for future use. Four ml of ACD:NS solution (see appendix 1B) was added to the pellet and the platelets were gently resuspended with a beryl transfer pipette. The platelet suspension was replaced in the centrifuge and spun at 1400g for 14 minutes to again pellet the platelets. This procedure of platelet washing with

ACD:NS solution was undertaken to remove any transferrin which would preferentially bind the Indium over the platelets (Hasain et al, 1969, Scheffel et al, 1977). The supernatant was then discarded without disturbing the platelet pellet. Five ml of ACD:NS solution were added and the platelets were again resuspended using a beryl pipette. The platelet suspension was then ready to be radiolabelled.

^{111} Indium oxine, 400µCi, was drawn up in a Hamilton syringe and added to the platelet suspension. This mixture was agitated and then allowed to sit at room temperature for twenty minutes. A longer incubation time did not improve Indium uptake and could potentially damage the platelets (Sinzinger et al, 1974). At the end of the incubation period, 1ml of PPP was added and the suspension to bind any remaining free Indium. The resulting mixture was centrifuged at 1400g for 14 minutes. The supernatant was carefully removed in order to avoid disturbing the platelet pellet and then discarded. Four ml of PPP were then added and the platelets were resuspended as previously described. The suspension was recentrifuged at 1400g for 14 minutes. The supernatant was again removed leaving the platelet pellet undisturbed. The platelets were washed with plasma as described above to remove any free or unbound

Indium prior to injection (Hasain et al, 1969, Scheffel et al, 1977). The platelets were then resuspended in 5ml of PPP. 10µl of the suspension was set aside for determination of the amount of radioactivity injected. The overall labelling process was approximately 2.5 hours in duration.

The labelled platelets were slowly reinjected through an 18 gauge needle into a forelimb vein. One hour after the injection a sample of blood was drawn from the opposite forelimb. The count rate of this sample was determined to estimate the amount of labelled platelets remaining in the circulation and by inference the amount of platelets damaged in the labelling process and removed by the reticuloendothelial system.

Prostacyclin Study

Prior to radiolabelling the platelets, 36 adult mongrel dogs were assigned group designations for the following day. Six equal groups were used (Table 1). Table 2 represents the demographic characteristics of each group.

Group 1 Control: These animals received an injection of normal saline (0.075ml/kg) into the right ventricle. One hour after the saline administration an infusion of 50mM tris buffer, pH 11 (see appendix 1C) was started at 5ml/hr and continued for five hours.

Group 2 Oleic Acid: 0.075ml/kg of 99% pure oleic acid was injected into the right ventricle. One hour later an infusion of 50mM tris buffer was commenced and continued at 5ml/hr (250μMol/hr) for five hours.

Group 3 Oleic Acid and Prostacyclin: Oleic acid was administered as described above. One hour after the injection an infusion of prostacyclin (100ng/kg/min) in 50mM of tris buffer was started. This infusion was five hours in duration.

Table 1 - Summary of Group Designations

Group Number	n	RV Injection	Infusion 1hr postRV injection
1	6	saline	tris buffer vehicle
2	6	oleic acid	tris buffer vehicle
3	6	oleic acid	tris buffer vehicle + PGI_2 (100ng/kg/min)
4	6	oleic acid	tris buffer vehicle + aminophylline (6mg/kg bolus and 0.4mg/kg/min infusion)
5	6	oleic acid	tris buffer vehicle + PGI_2 (100ng/kg/min) + aminophylline (6mg/kg bolus and 0.9mg/kg/min infusion)
6	5	saline	tris buffer vehicle + PGI_2 (100ng/kg/min)

Table 2 - Demographic Data of Dogs

	Weight		Age	
Group Number	mean(kg)	Range	mean(yrs)	Range
1	23.4	19.3 - 30.4	2.1	0.7 - 4
2	23.8	21.7 - 27.0	2.1	0.8 - 3
3	22.2	18.2 - 25.4	1.5	1 - 2
4	23.2	20.6 - 25.6	2.6	0.7 - 4
5	21.61	18.5 - 26.5	2.4	0.8 - 4
6	22.7	20.0 - 25.4	2.4	1 - 7

Group 4 Oleic Acid and Aminophylline: Oleic acid was administered as previously mentioned. One hour later, a loading dose of aminophylline (6mg/kg) was injected followed by an infusion of 0.9mg/kg. As well 50mM tris buffer was also infused commencing one hour after oleic acid.

Group 5 Oleic Acid, Prostacyclin and Aminophylline: One hour after the oleic acid injection both prostacyclin and aminophylline were administered as previously described.

Group 6 Control and Prostacyclin: Saline was injected as previously described. One hour later an infusion of prostacyclin, as described above, was started. The prostacyclin was given over five hours.

Due to the unstable nature and high cost of prostacyclin, dogs receiving this drug were studied in batches and not in random order. However, dogs were selected randomly, based on weight criteria of 18-25kg, by an independent person unaware of the nature of the study. The dog was assigned to its group before selection to avoid bias as much as possible.

Each dog was kept isolated in a separate room from all other animals. Water was allowed ad libitum but food was withheld on the day of the prostacyclin trial.

General anesthesia was induced with an intravenous injection of pentobarbital (30mg/kg). Supplemental doses of 30 to 60mg were given hourly. The supplementation could be repeated once or twice within the hour as required. The trachea was intubated after induction of anesthesia and the animals were placed on intermittent positive pressure ventilation (Harvard 607 constant volume ventilator). Tidal volume was set at 10ml/kg and the respiratory rate was adjusted to keep the $PaCO_2$ between 33 and 38 torr. Airway pressure was measured using a Statham PM5 transducer and recorded on a Gould 2600S strip chart recorder. End tidal carbon dioxide concentration was measured using a capnograph (Godart Type 146).

Access to the left femoral vessels was gained by cutdown. These vessels were exposed with blunt dissection. The femoral vein was cannulated first with PE200 polyethylene tubing (Intermedic). The catheter was then advanced into the inferior vena cava and infusion of maintenance fluids commenced. Approximately 15ml/kg/hr of intravenous fluids, consisting of equal parts normal saline and 5% dextrose in water, were required to

maintain adequate urine outputs, prevent hemoconcentration and keep the pulmonary artery wedge pressure and cardiac output at control values. The femoral artery was then cannulated with PE 200 tubing and the catheter was advanced into the distal aorta. The arterial catheter was connected to a Statham P23db pressure transducer and the pressure wave form displayed on the strip chart recorder. The arterial catheter was also used for blood sampling.

Electrocardiograph leads were attached with 22 gauge needles. Lead placement included right and left forelimbs and cardiac apex. The electrocardiogram was also monitored on the strip chart recorder.

Next, the right internal jugular was cannulated percutaneously and a 6 Fr catheter sheath introducer (Cordis) inserted. A 5 Fr pediatric pulmonary artery catheter (Swan-Ganz, Edwards Laboratories) was floated into the wedge position under pressure wave form guidance. Pressure measurements were taken on a Statham P23db pressure transducer and monitored on the strip chart recorder. The pulmonary artery catheter allowed access to the central circulation for mixed venous blood sampling. Mixed venous blood was drawn only when an undamped pulmonary artery pressure signal was obtained on the strip chart recorder.

All transducers were electronically zeroed at the level of the midchest. The two Statham P23db transducers were calibrated against a mercury manometer while the Statham PM5 transducer was calibrated against a water manometer. All pressure measurements were made at the end of expiration.

Cannula insertion required approximately 45 minutes. The dogs were allowed a further 30 minutes to stabilize before the control measurements were made. These included physiological parameters such as heart rate, core temperature, cardiac output, end-tidal carbon dioxide concentration, systemic arterial, pulmonary artery, pulmonary wedge, central venous and airway pressures. As well, blood was withdrawn for measurment of hemoglobin concentration, arterial and mixed venous $P0_2$, $PC0_2$, pH, platelet count and disintegrations per minute as an estimate of radioactivity.

Temperature was measured by a thermistor in the pulmonary artery catheter. Cardiac output was measured using the thermal dilution technique. Three ml of 5% dextrose in water at 0°C were injected rapidly at end expiration. The mean of three determinations which were within 10% of each other was used and the calculations were made by an on-line microprocessor (9520A Cardiac Output Computer, Edwards Laboratories).

Three mls of blood were collected in a heparinized syringe from both the indwelling arterial and pulmonary artery catheters. These samples were analyzed separately for pH, $P0_2$ and $PC0_2$ with a blood gas analyzer (IL 113, Instrument Laboratories). The blood gas and pH electrodes were calibrated against gas standards and two known pH standards prior to every measurement. Blood (6 ml) was also obtained for arterial and venous platelet counts. These samples were collected and stored in manufactured EDTA air evacuated test tubes (Vacutainer, Becton Dickinson). The samples were analyzed in an automatic platelet counter (PL100, TOA). One ml of arterial and venous blood was set aside for count rate determination. Measurements were made in a gamma counter (Nuclear Chicago Model 1185). The background radiation was subtracted from the sample counts. Finally, hemoglobin was measured in a hemoglobinometer (HGBR2, Coulter Electronics) using a modified ferricyanide technique (Wintrobe 1981).

After the control measurements and samples were obtained, the pulmonary artery catheter was withdrawn into the right ventricle. Catheter position was verified by the pressure waveform on the chart recorder. At this point, 0.075ml/kg of oleic acid or normal saline, depending upon the group designation of the dog, was

injected over ten seconds into the right ventricle. At the end of the injection, the catheter was flushed with 3ml of heparinized normal saline and returned to its position in the pulmonary artery. Just prior to the right ventricular injection, the animals were taken off the ventilator and allowed to breathe spontaneously. Spontaneous respiration was continued during the injection and for five minutes afterwards to allow for natural distribution of the oleic acid or saline throughout the pulmonary vascular tree, free of any artifact induced by positive pressure ventilation. At the end of the five minute period, the animals were returned to intermittent positive pressure ventilation.

Measurement of all hemodynamic parameters such as cardiac output, heart rate, arterial, pulmonary artery, wedge and central venous pressures were repeated at one half hour after the right ventricular injection of oleic acid or saline and hourly thereafter for five hours. Airway pressure, end tidal carbon dioxide concentration, temperature and arterial and venous blood gases were measured concurrently with the hemodynamic parameters. Arterial and venous indium^{111} count rate determinations were sampled at 0.5, 1, 2, and 5 hours after the injection of oleic acid or saline while arterial and venous samples for platelet counts were drawn at 0.5,

1 and 5 hours after the right ventricular injection. Finally, hemoglobin was measured 4 hours after the right ventricular injection to make certain hemoconcentration had not occurred and repeated at 5 hours for the final calculations (see Table 3 for times and frequency of measurements and samples).

One hour after the right ventricular injection, an infusion of 50mM Tris buffer pH (10.5), prostacyclin (100mg/kg/min) in Tris buffer and/or aminophylline (0.9mg/kg/hr) which was preceded by a bolus of 6mg/kg were commenced depending on the group to which the dog was assigned (Table 1). The infusion was carried out over 4 hours. Prostacyclin was diluted with 50mM pH 10.5. Tris buffer was infused via a Harvard peristaltic pump. The diluted drug was placed into a plastic syringe and continuously cooled with ice water. New batches of drug were prepared and infused every two hours to prevent loss of prostacyclin potency.

Five hours after the right ventricular injection, the chest was opened using a left parasternal incision. The dogs were then sacrified with an overdose of pentobarbital. The hilum of the left lung was rapidly clamped and the lung was removed from the chest. The left lower lobe was placed on the counter in the position it would be found in the supine animal. It was then sectioned coronally into three portions designated

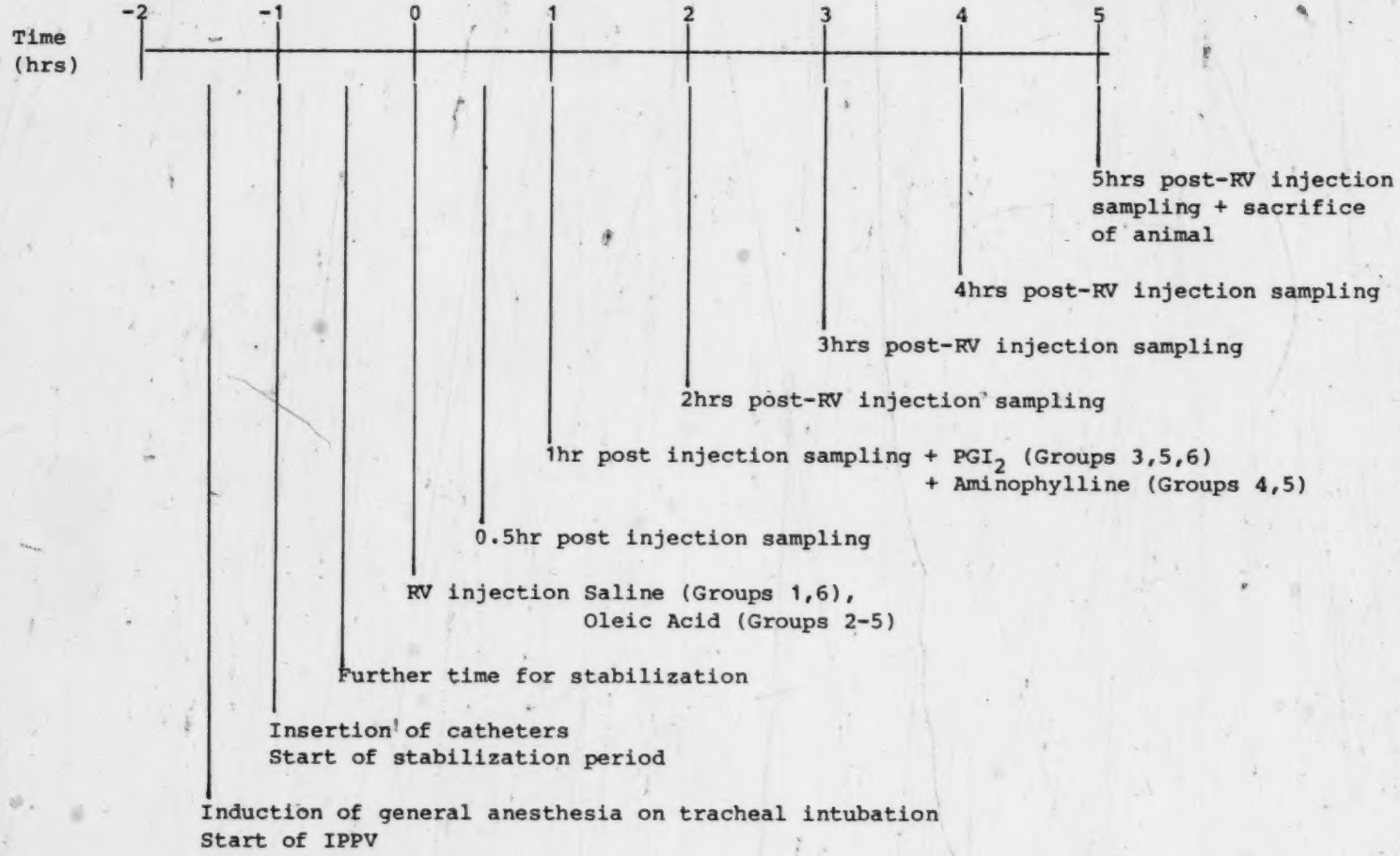
Table 3 - Measurement and Blood Sampling

		Post-RV Injection					
Parameter	Control	0.5	1	2	3	4	5
Hemodynamics	X	X	X	X	X	X	X
Respiratory	X	X	X	X	X	X	X
Blood Gases	X	X	X	X	X	X	X
Blood Radioactivity	X	X	X	X			X
Platelet Counts	X	X	X				X
Hemoglobin	X					X	X

ventral, middle and dorsal lung zones. With the animal in the supine position these portions correspond to the physiological upper, middle and lower lung zones, respectively. A sample from each section was placed in a preweighed test tube for count rate and tissue hemoglobin content determinations. The remaining portions of left lower lobe were put in separate containers and weighed, thus determining the wet weight. The portions were then placed in a drying oven (Model 287-A, Dispatch Oven Company) and removed daily for weighing. The dry weight was determined when the weight varied less than 2% from the previous day. Samples from liver, spleen and gracilis muscle were also placed in preweighed test tubes for count rate and tissue hemoglobin content determinations. All weights were obtained on a Mettler balance model P1000. (See Figure 2 for time course of the experiment.)

Samples of tissue for hemoglobin content were ground in a Virtis 45 homogenizer. 20µl of this solution was placed in a 5ml aliquot of Drabkins solution (see Appendix 1F). This mixture was allowed to stand for 5 minutes. Optical density was then measured in a colorimeter (Klett Summerson, Model 800-3) (Drabkin et al 1932). The measurement obtained was multiplied by an appropriate calibrating factor to produce tissue

Figure 2 - Sequence of Events



hemoglobin concentration. Accuracy of this technique was verified with a Coulter hemoglobinometer. With the tissue hemoglobin concentration known, the tissue blood volume was calculated on the assumption that all hemoglobin remained in the intravascular space.

The right lung was carefully removed and inflated with 10% formalin to a pressure of 20 cm H_2O. The inflation pressure was maintained for 48 hours with a standing column of formalin using a roller pump. Sections were then taken from corresponding upper, middle and lower lung zones and placed awash in formalin. These sections were fixed and stained with hematoxylin and eosin for light microscopy. The slides were set aside and examined at a later date and graded for pulmonary damage.

Table 4 - Source of Drugs and Chemicals

Acepromazine	(Atrevet)	Ayerst Laboratories, Montreal, PQ
^{111}Indium Oxine		Mediphysics, Emeryville, CA
Prostacyclin	Lot # 33F-08731	Sigma Chemical Co, St. Louis, MO
	Lot # 945 U-A	Upjohn, Kalamazoo, MICH
Aminophylline		Glaxo Laboratories,

Calculations

1) Oxygen saturations were calculated from PO_2, pH and body temperature using the equation developed by Rossing and Cain. The equation is a mathematical estimation of oxygen-hemoglobin disassociation curve in dogs and has been derived from 591 blood samples.

$$\log \left(S/1-S\right) = 2.5198 \log PO_2 + 1.1804 (pH-7.0) - 0.47234T - 2.2621,$$

where; S represents the saturation expressed as a decimal fraction,

PO_2 represents the partial pressure of oxygen in mmHg,

and, T represents the temerature in degrees centigrade (Rossing and Cain, 1966).

2) Alveolar partial pressure of oxygen was calculated from a simplification of the alveolar air equation developed by Nunn (1977),

$$PAO_2 = P_IO_2 - \frac{PaCO_2}{R},$$

where; PAO_2 being the partial pressure of oxygen in mm Hg in the alveolus,

P_IO_2 being the inspired partial pressure of oxygen,

$PaCO_2$ being the partial pressure of carbon dioxide in the arterial blood and thus a rough estimation of the partial pressure of carbon dioxide in the alveolus,

and, R representing the respiratory quotient which was estimated at 0.8 for these studies. In animals or humans who have fasted, the R is usually measured between 0.7-0.8. An R of 0.8 is suggested in anesthetized man although this has yet to be measured.

3) Arterial, venous and capillary blood oxygen contents were calculated by the product of the respective saturations, the hemoglobin concentrations and the oxygen combining capacity of hemoglobin at 1.34ml/gm hemoglobin (Starling 1912). While pure hemoglobin has been estimated to carry 1.39ml of oxygen per gram, most blood is contaminated with methemoglobin or carboxyhemoglobin. The concentration of contaminants is approximately 4% thus reducing oxygen carriage from 1.39ml/gm to 1.34ml/gm (Guyton 1981). As well, the method of oxygen extraction may produce additional error in this value. For example:

$$CaO_2 = SaO_2 \times [Hgb] \times 1.34,$$

where; CaO_2 represents the arterial blood oxygen content in ml per 100ml of blood,

$Sa0_2$ represents the arterial oxygen saturation expressed as a fraction,

and, [Hgb] represents the hemglobin concentration. The amount of oxygen physically dissolved in plasma was ignored in these calculations as all experiments were conducted during room air breathing and this value is negligible.

4) Calculated venous admixture was also obtained. This term is used instead of shunt fraction as measurements were made inspiring room air (Nunn 1977). This value is expressed as a fraction of total blood flow so that;

$$\dot{Q}s/\dot{Q}t = \frac{Cc'0_2 - Ca0_2}{Cc'0_2 - C\bar{v}0_2},$$

where; $\dot{Q}s/\dot{Q}t$ is the calculated venous admixture,

$Cc'0_2$ is the oxygen content of the alveolar capillary blood,

$Ca0_2$ is the oxygen content of the arterial blood,

and, $C\bar{v}0_2$ is the oxygen content of the mixed venous blood.

Because all experiments were done utilizing room air, the effect of dissolved oxygen was ignored. As $Pa0_2$ was not greater than 90mmHg, the error produced by the manoeuver is less than 1%.

5) Oxygen consumption was calculated using the Fick equation;

$$\dot{V}O_2 = \dot{Q}(CaO_2 - C\bar{v}O_2),$$

where; $\dot{V}O_2$ represents oxygen consumption,

$\dot{Q}$ represents the cardiac output calculated by the thermodilution technique,

CaO_2 represents the arterial oxygen content,

and, $C\bar{v}O_2$ represents the mixed venous oxygen content.

6) The alveolar arterial partial pressure gradient for oxygen was also calculated inspiring room air. This gives the equation:

$$A\text{-}aDO_2 = 0.21\ (P_B - 47) - \frac{PaCO_2}{R} - PaO_2,$$

where; $A\text{-}aDO_2$ is the alveolar arterial partial pressure gradient for oxygen,

P_B is the barometric pressure measured for each experiment,

$PaCO_2$ is the arterial partial pressure of carbon dioxide,

R is the respiratory quotient,

and, PaO_2 is the arterial pressure pressure of oxygen.

Statistical Analysis

Means and stardards deviations were calculated for all measured parameters and derived values. In order to compare the results of this study to others, groups 2 (oleic acid), 3 (oleic acid plus prostacyclin), 4 (oleic acid plus aminophylline) and 5 (oleic acid, prostacyclin and aminophylline) were compared to each other by analysis of variance. As well, Group 1 was compared to groups 2 and 6 independently and again tested with analysis of variance. All parameters were also compared with respect to the change over time from their control value to the 5 hour postoleic acid value and examined with a paired t-test. Probability values of $p<0.05$ were considered to be statistically significant.

RESULTS

The results will be divided and presented in four major categories: hemodynamic measurements, blood sampling results, derived hemodynamic parameters and pulmonary pathology.

Hemodynamic Measurements

i) Heart Rate (Table 5a): all groups with the exception groups 4 and 6 showed a nonsignificant but general progression of increasing heart rate throughout the study period. However, these increases were small and the interanimal variation in heart rate was large. Of interest, group 5 (oleic acid, prostacyclin and aminophylline) demonstrated a statistically significant rise in ($p<0.02$) when compared to that group's control value. The rise in heart rate appeared to correspond to the time period one hour after oleic acid adminstration. That is, at the commencement of prostacyclin and/or aminophylline infusions. Groups 1 (control) and 6 (prostacyclin alone) showed little change in heart rate throughout the experimental period. The prostacyclin groups (3 and 5) tended towards a greater heart rate than any of the other groups.

ii) Mean Arterial Pressure (Table 5a, Figures 3,4): there was no change in mean arterial pressure (MAP) in

groups 1 (control), 2 (oleic acid), 4 (oleic acid and aminophylline) and 6 (prostacyclin alone); however, groups 3 (oleic acid and prostacyclin) and 5 (oleic acid, prostacyclin and aminophylline) demonstrated a marked drop in MAP after the prostacyclin infusion was commenced. Not only was the fall in blood pressure significant when compared to each individual group's control value ($p<0.05$) but also significant ($p<0.02$) when compared to the nonprostacyclin groups (2 and 4).

iii) Cardiac Output (Table 5a, Figures 5,6): groups 2 (oleic acid) and 4 (oleic acid and aminophylline) demonstrated a steady and statistically significant decline in cardiac output ($p<0.01$ group 2, $p<0.05$ group 4) after oleic acid injection. However cardiac output in groups 3 (oleic acid and prostacyclin) and 5 (oleic acid, prostacyclin and aminophylline) was maintained at the control value or even rose slightly during the trial period. The difference between the non-prostacyclin groups 2 and 4 and the prostacyclin groups 3 and 5 was significant ($p<0.01$ at 3, 4 and 5 hours postoleic acid injection) when cardiac output was compared. This trend emerged one hour after the prostacyclin infusion was commenced and continued for the duration of the 5 hour experiment. No significant changes were seen in cardiac output in group 1 (controls) and group 6 (prostacyclin only).

iv) Pulmonary Artery Pressure (Table 5b): in all groups, with the exception of group 6 (prostacyclin only), the pulmonary artery pressure (PAP) rose significantly when compared to each of the individual control values. This trend was also seen in group 1 (controls) and although considered significant, the rise was not as large as any seen in the oleic acid groups (2,3,4 and 5). While the trend consistently showed group 2 (oleic acid) PAP values to be greater than those of group 1 (controls), the difference was only statistically significant at 5 hours postoleic acid injection ($p<0.02$). As well, Groups 3 (oleic acid and prostacyclin) and 4 (oleic acid and aminophylline) demonstrated a smaller rise in PAP when compared to the animals given oleic acid alone (Group 2). This trend was not significant statistically. In other words, prostacyclin or aminophylline tended to blunt the rise in PAP. PAP in group 5 (oleic acid, aminophylline and prostacyclin) was significantly lower than that of any other oleic acid group at 4 and 5 hours after the oleic acid injection. Perhaps a clear trend did not emerge because of a wide standard deviation. No change in PAP was seen in group 6 (prostacyclin only).

v) Peak Inspiratory Pressure (Table 5b): while peak inspiratory pressure (PIP) rose in all groups over the

duration of the trial, the rise was significant statistically in groups 3 (oleic acid and prostacyclin), 4 (oleic acid and aminophylline), 5 (oleic acid, aminophylline and prostacyclin) and 6 (prostacyclin only) when compared to that individual group's control value. Intergroup differences were not observed.

vi) Wedge and Central Venous Pressures: no significant changes were seen in either wedge and central venous pressures throughout the trial in any group. As well, no intergroup differences were noted.

Blood Sampling Results

i) Arterial PO_2 (Table 5c): all groups given oleic acid (groups 2,3,4 and 5) demonstrated a decreased arterial PO_2 (PaO_2) with time. The fall was maximal at 2 hours after the oleic acid injection and thereafter the PaO_2 remained relatively constant. No intergroup differences were seen within the oleic acid groups. The fall in PaO_2 was significant ($p<0.05$ groups 2 and 3, $p<0.02$ groups 4 and 5) when compared to the individual control value for this parameter. There was obviously a statistically significant difference when the PaO_2 of control animals (group 1) was compared to that of oleic acid animals (group 2). The control group demonstrated no change in PaO_2 throughout the duration of the drug trial. Of

interest, group 6 (prostacyclin only) revealed a rise in $Pa0_2$ throughout the course of the trial.

ii) Mixed Venous $P0_2$ (Table 5c): again, all groups given oleic acid showed a progressive fall in mixed venous $P0_2$ ($\bar{P}v0_2$) throughout the entire duration of the study. This fall was statistically significant ($p<0.01$ for groups 2 and 4, $p<0.05$ for groups 3 and 5) when compared to that individual's control value. While the fall in $\bar{P}v0_2$ appeared to be greater in the non-prostacyclin groups (2 and 4) than the prostacyclin groups (3 and 5), no significant differences were seen between the groups. As expected, the control group (1) $\bar{P}v0_2$ remained unchanged throughout the study as did that of group 6 (prostacyclin only).

iii) Arterial Oxygen Saturation (Table 5c): arterial oxygen saturation was also calculated and compared. Due to a large variance in this parameter, analysis did not produce any meaningful results. Generally speaking, arterial oxygen saturation fell with time after oleic acid administration. However, the fall was only statistically significant in groups 2 (oleic acid), 4 (oleic acid and aminophylline) and 5 (oleic acid, prostacyclin and aminophylline) when compared to the preoleic acid control values for each group. No significant intergroup differences were demonstrated and

this included differences between control animals (group 1) and those given oleic acid (group 2).

Again, groups 1 and 6 revealed no change in saturation over the time course of the trial.

iv) Mixed Venous Oxygen Saturation (Table 5d): blood mixed venous oxygen saturation exhibited a trend similar to arterial saturation. That is, a significant fall in mixed venous saturation in groups 2 (oleic acid), 4 (oleic acid and aminophylline), and 5 (oleic acid, aminophylline and prostacyclin) when compared to each group's control value. All groups with the exception of group 6 (prostacyclin only) demonstrated a fall in mixed venous saturation; however, no significant intergroup differences occurred.

v) Arterial and Venous Platelet Counts (Table 5e): no significant trend emerged when studying these parameters. As well, no intergroup differences were seen.

vi) Arterial Indium^{111} Count Rate (Table 5e): while arterial blood count rate tended to fall with time in all groups, a wide variation make the results difficult to interpret. The fall in arterial blood count rate was significant in groups 2 (oleic acid), 3 (oleic acid and prostacyclin), 5 (oleic acid, prostacyclin and aminophylline) and 6 (prostacyclin only) when compared to

each group's control value. Because of a large standard deviation in groups 1 (controls) and 4 (oleic acid and aminophylline), the fall in blood count rate while comparable to other groups, was not significant statistically. No differences were seen between blood count rate of control animals (group 1) and that of those given oleic acid (group 2). Finally, groups 3 (oleic acid and prostacyclin) and 5 (oleic acid, prostacyclin and aminophylline) appeared to have a greater reduction in blood count rate than the non-prostacyclin groups (2 and 4) $p<0.05$.

vii) Venous Blood Indium^{111} Count Rate: while all groups demonstrated a trend toward falling venous blood count rate, this fall was only significant in group 6. Secondly, no statistically significant intergroup differences appeared. However, the decrease in venous blood count rate was less than that observed in arterial blood in all groups except group 6 (prostacyclin only).

viii) Hemoglobin: no changes in hemoglobin over time, within each group or between groups, were seen.

Derived Hemodynamic Parameters

i) Systemic Vascular Resistance (Table 5a, Figures 7,8): as systemic vascular resistance (SVR) was calculated from cardiac output and MAP, the trend for this parameter was

similar to that previously described for both cardiac output and MAP. That is, a significant increase was seen in SVR in the non-prostacyclin groups (2 and 4) when compared to their respective preoleic acid control values. As well, SVR was significantly lower ($p<0.02$) for the prostacyclin groups (3 and 5) when compared to the nonprostacylin groups (2 and 4). No significant changes were seen in groups 1 (controls) and 6 (prostacyclin) with respect to SVR.

ii) Pulmonary Vascular Resistance (Table 5b): this parameter presents a confusing picture of data with no consistent trend emerging. While pulmonary vascular resistance (PVR) rose in all groups except group 6 (prostacyclin only), the rise was only statistically significant in groups 2 (oleic acid), 4 (oleic acid and aminophylline) and 5 (oleic acid, aminophylline and prostacyclin). At 3 and 4 hours after oleic acid administration, the PVR was significantly lower in the prostacyclin groups (3 and 5) when compared to the nonprostacyclin groups (2 and 4). While the previously mentioned trend continued at 5 hours postoleic acid injection, it was not statistically significant at that time. However, the standard deviations of all groups were large. The only exception to the previously

mentioned trend was group 6, where the PVR actually fell slightly.

iii) Stroke Volume (Table 5b): while stroke volume (SV) fell acutely after oleic acid administration and remained depressed for the rest of the experiment, the fall was only significant in group 2 when compared to the preoleic acid control values. As well, the fall in SV in the prostacyclin groups (3 and 5) was less than the nonprostacyclin groups (2 and 4) at 3 and 4 hours after oleic acid administration ($p<0.05$). Naturally, no changes were seen throughout the experiment in the two groups receiving saline instead of oleic acid, groups 1 and 6.

iv) Alveolar-Arterial Oxygen Tension Gradient (Table 5c): the alveolar-arterial oxygen tension gradient ($A-aDO_2$) rose dramatically after oleic acid adminstration peaking 3 to 4 hours later. All groups given oleic acid (groups 2,3,4, and 5) demonstrated the aforementioned trend. This finding was considered statistically significant in all groups when compared to each group's preoleic acid value ($p<0.05$). However, no intergroup differences were seen between the animals receiving oleic acid. Naturally, a significant difference in $A-aDO_2$ occurred when groups 1 (controls) and 2 (oleic acid) were compared. This difference started 0.5 hour after oleic

acid adminstration and continued for the duration of the trial. As expected, no change in A-aDO_2 occurred in the groups receiving saline instead of oleic acid (groups 1 and 6). However, the variation in the groups receiving oleic acid was extremely large.

v) Calculated Venous Admixture (Table 5d): again, all groups receiving oleic acid demonstrated a rise in calculated venous admixture ($\dot{Q}_S/\dot{Q}_T$) commencing 0.5 hours after oleic acid adminstration. This rise was only statistically significant in groups 3,4 and 5 when compared to each group's preoleic acid value. There was obviously no change in $\dot{Q}_S/\dot{Q}_T$ in both groups receiving saline instead of oleic acid (groups 1 and 6). When the control animals (group 1) were compared to those given oleic acid (group 2), a marked difference emerged commencing 0.5 hours after the right ventricular injection of oleic acid or saline. Another interesting observation was a slight increase in $\dot{Q}_S/\dot{Q}_T$ in groups 3,4 and 5 when they were compared to group 2 (oleic acid only). This increase was statistically significant ($p<0.05$) at 2 and 4 hours postoleic acid adminstration. Again, as expected, groups 1 and 6 demonstrated no change in $\dot{Q}_S/\dot{Q}_T$ throughout the experimental period.

vi) Arterial Venous Oxygen Content Gradient (Table 5d): no consistent trend emerged when the arterial-venous oxygen content gradient ($A\text{-}VDO_2$) was studied. Unfortunately, these values demonstrated a large standard deviation. There was a tendency for the prostacyclin infused groups (3 and 5) to have a slightly lower $A\text{-}VDO_2$ than those not given prostacylcin.

vii) Oxygen Consumption (Table 5d): again, no consistent trend emerged when this parameter was studied. A large degree of variation in results occurred, obscuring the expression of any possible underlying trends.

Pulmonary Pathology

i) Gross Pathology: while the lungs of animals receiving saline (groups 1 and 6) instead of oleic acid were not grossly abnormal, the lungs of the oleic acid groups (2,3,4, and 5) were obviously abnormal. The least dependent lung regions of these groups appeared to be relatively spared of injury. The middle zones showed patchy areas of hemorrhage and edema which became confluent in the dependent lung regions. The previously described areas each corresponded to roughly one third of the lung volume. The pattern was obviously gravitational with anterior (superior) portions of lung being fairly

well preserved, while the posterior (dependent) portions were most adversely affected. Finally, foam, indicating alveolar edema, exuded from airways in all animals given oleic acid regardless of treatment group.

ii) Microscopic Pathology: generally speaking, few abnormalities could be demonstrated with the microscopic examination of lungs from dogs given saline instead of oleic acid. Histological abnormalities were limited to one or two focal microscopic areas of hemorrhage or edema. These areas were probably artifacts induced by the pulmonary artery catheter. Animals receiving oleic acid developed a patchy hemorrhagic pneumonia which became confluent in dependent lung zones. Histological findings ranged from minimal abnormality, septal and interstitial edema, alveolar flooding to hemorrhagic infiltration. There appeared to be a gradient in the severity of the pulmonary lesion with nondependent areas showing more benign or normal pathology to severe diffuse hemorrhagic infiltration in the dependent pulmonary regions. The various treatment modalities could not be differentiated from the pathological examination.

iii) Wet/Dry Ratios (Table 5f, Figure 9): a marked rise in wet/dry ratios occurred in the animals given oleic acid ($p<0.02$). However, no differences could be shown between any of the oleic acid groups. As expected, no

difference in wet/dry ratio occurred between the two non-oleic acid groups (1 and 6).

iv) Lung Indium^{111} Uptake (Table 5f, Figure 10): the uptake of radiolabelled platelets by the lung exhibited a similar pattern as described for wet/dry ratios. A significant difference was obtained between the control animals (Group 1) and those given oleic acid (Group 2) $p<0.02$. However, no differences occurred between any of the oleic acid groups (2,3,4, and 5). Again, the values for indium uptake in the two saline groups (1 and 6) was exactly the same.

v) Calculated Pulmonary Residual Blood Volume (Table 5f, Figure 11): examination of this value suggested a trend towards reduction in residual blood volume occurred in the oleic acid groups (2,3,4 and 6) when compared to the controls (group 1). This change, however, was not significant. As well, it appeared that the prostacyclin groups (3 and 5) had a greater fall in residual blood volume than did the nonprostacyclin groups (2 and 4) $p<0.05$. However, no difference could be demonstrated between the two groups receiving saline instead of oleic acid (groups 1 and 6).

Finally blood volume of peripheral tissues such as liver, spleen and muscle showed a wide interanimal

variation with no consistent trend emerging between the various treatment groups. As a result, this data has not been reported.

Table 5a - Mean and Standard Deviation of All Parameters Measured over the 5 Hour Trial

Group	Control	0.5 post	1 post	2 post	3 post	4 post	5 post
Heart Rate - mean ± standard deviation							
1	160.5 ± 23.5	186.7 ± 14.3	173.3 ± 23.5	170.8 ± 15.9	170.8 ± 16.0	158.8 ± 30.4	164.2 ± 24.4
2	162.0 ± 33.0	178.0 ± 19.3	181.2 ± 17.8	165.0 ± 23.1	167.5 ± 27.3	175.3 ± 35.2	170.0 ± 35.1
3	148.3 ± 32.3	155.2 ± 31.0	166.3 ± 25.1	165.8 ± 23.7	168.0 ± 24.5	172.2 ± 21.6	171.3 ± 22.0
4	173.5 ± 39.1	169.7 ± 22.3	170.0 ± 25.5	173.3 ± 22.9	173.0 ± 24.9	166.0 ± 31.0	164.0 ± 32.2
5	133.0 ± 26.2	144.2 ± 18.6	159.2 ± 13.4	182.5 ± 9.01	178.3 ± 7.45	171.0 ± 11.1	170.0 ± 15.3**
6	159.0 ± 46.4	164.0 ± 42.6	166.0 ± 41.4	156.0 ± 39.2	153.0 ± 33.6	153.0 ± 27.1	159.0 ± 31.0
MAP							
1	127.3 ± 28.2	111.3 ± 14.5	121.0 ± 19.9	131.7 ± 19.4	127.0 ± 11.2	141.7 ± 13.9	130.6 ± 21.4
2	131.8 ± 14.7	123.8 ± 18.8	121.2 ± 29.2	136.6 ± 19.0	140.8 ± 18.9**	140.4 ± 16.4**	135.1 ± 16.1
3	132.7 ± 18.8	117.4 ± 20.1	115.7 ± 21.2	92.5 ± 13.6**	104.7 ± 14.6**	102.0 ± 18.1**	108.7 ± 10.9**
4	135.5 ± 21.4	123.2 ± 16.3	124.0 ± 21.1	133.9 ± 18.1	139.1 ± 7.6	142.1 ± 11.5	164.2 ± 32.2
5	126.1 ± 13.6	106.9 ± 22.0+	102.2 ± 17.1	85.0 ± 26.4**	90.1 ± 27.7**	80.2 ± 23.7**	92.1 ± 26.1**
6	137.1 ± 3.1	134.1 ± 7.4+	133.9 ± 2.8	124.9 ± 16.7	137.1 ± 16.3	134.2 ± 15.5	131.6 ± 16.5
$\dot{Q}_T$							
1	3.75 ± 0.62	4.78 ± 1.17	4.44 ± 1.07	4.17 ± 1.26	3.21 ± 1.08	4.15 ± 2.32	4.17 ± 1.74
2	4.81 ± 0.91	3.89 ± 1.04	3.87 ± 0.95	3.25 ± 0.86	2.79 ± 0.70	2.80 ± 0.72	3.03 ± 0.75***
3	4.66 ± 0.67	4.64 ± 0.78	4.27 ± 0.87	4.02 ± 0.38*	4.09 ± 0.47**	4.65 ± 0.76**	5.06 ± 0.51**
4	4.54 ± 1.06	3.66 ± 0.55	3.52 ± 0.54	3.13 ± 0.42	2.95 ± 0.60	2.89 ± 0.04	3.06 ± 0.74*
5	3.85 ± 0.75	3.94 ± 0.62	3.86 ± 0.70	4.05 ± 0.60*	4.00 ± 0.63**	3.93 ± 0.56**	4.13 ± 1.03**
6	4.00 ± 1.12	4.01 ± 0.86	4.07 ± 0.84	4.08 ± 1.07	3.84 ± 1.26	3.95 ± 1.24	4.40 ± 1.06
SVR							
1	2887.3±1021.8	2070.7± 764.2	2311.3± 789.4	2765.0±1068.8	2245.0± 613.9	3594.4±1858.1	3221.3±1430.5
2	2253.7± 384.4	2870.2±1265.6	2773.0±1291.9	3775.2±1667.0	4556.5±2148.5	4390.3±2053.7	3961.0±1656.2*
3	2309.8± 233.1	2036.8± 239.1	2212.0± 505.8	1872.5± 363.5**	2045.7± 317.2**	1754.5± 192.3**	1709.5± 215.7**
4	2440.0± 259.6	2748.8± 305.4	2888.3± 503.4	3507.7± 664.3	3887.8± 468.0	4054.3± 642.5	3931.3±1122.5*
5	2745.0± 664.7	2240.7± 621.3	2199.2± 587.6	1743.3± 719.4**	1893.7± 752.9**	1668.4± 636.0**	1856.2± 708.3**
6	3012.0± 925.2	2824.6± 677.3	2798.2± 601.2	2712.2± 768.3	3167.2±1069.1	2948.0± 954.4	2523.2± 681.4

Table 5b

Group	Control	0.5 post	1 post	2 post	3 post	4 post	5 post
PAP							
1	10.5 ± 1.7	8.8 ± 0.8	10.5 ± 1.7	13.4 ± 4.5	14.6 ± 6.1	16.3 ± 4.2	17.0 ± 3.6°
2	13.8 ± 4.5	12.4 ± 2.6	13.8 ± 4.5	15.5 ± 3.1	17.8 ± 3.6	21.5 ± 3.7	25.0 ± 3.7°°°II
3	15.1 ± 5.5	16.8 ± 6.2	15.1 ± 5.5	17.2 ± 7.9	16.7 ± 5.9	19.1 ± 6.2	22.3 ± 7.0
4	11.6 ± 3.6	11.4 ± 2.6	11.6 ± 3.6	12.7 ± 3.6	14.3 ± 4.4	16.3 ± 4.9	19.2 ± 6.3°
5	11.1 ± 1.8	12.2 ± 1.1	11.1 ± 1.8	10.9 ± 0.8	12.4 ± 2.0	12.7 ± 1.8*	15.3 ± 2.6°°*
6	13.1 ± 2.6	11.9 ± 3.4	13.1 ± 2.6	12.7 ± 2.1	13.0 ± 2.3	13.9 ± 2.2	13.5 ± 2.7
PVR							
1	185.4 ± 36.4	132.5 ± 14.0	174.3 ± 61.4	215.7 ± 80.5	172.0 ± 44.5	308.3 ± 15.5	300.9 ±199.3
2	186.1 ± 58.0	203.2 ± 52.6	209.8 ± 21.0	280.0 ± 41.6	394.6 ± 79.6II	408.2 ± 68.7I*	438.2 ±135.3°
3	197.1 ± 42.8	211.5 ± 71.3	255.7 ± 85.2	289.7 ±121.3	255.7 ± 81.0*	275.0 ±118.9*	290.0 ± 90.5
4	172.0 ± 28.4	225.6 ± 93.4	219.3 ± 66.2	261.2 ± 86.3	345.4 ±107.1	384.4 ±118.3	465.4 ±213.2°
5	175.3 ± 39.7	209.4 ± 50.5	208.1 ± 41.1	199.5 ± 24.5	214.5 ± 68.0*	249.2 ± 29.5*	284.7 ± 63.4°°°
6	215.8 ± 87.3	154.2 ± 42.1	171.3 ± 42.9	191.8 ± 37.0	193.4 ± 36.9	182.6 ± 38.4	164.7 ± 43.2
PIP							
1	16.8 ± 7.4	11.2 ± 3.3	16.6 ± 9.3	17.1 ± 8.5	13.6 ± 3.5	17.8 ± 8.9	18.4 ± 8.5
2	11.9 ± 2.1	14.5 ± 3.8	15.7 ± 6.1	17.3 ± 6.7	19.4 ± 10.6	19.6 ± 10.5	18.5 ± 10.7
3	11.4 ± 1.3	13.1 ± 1.7	14.3 ± 2.0	14.9 ± 2.3	15.3 ± 3.3	15.4 ± 3.1	17.6 ± 5.9°
4	16.3 ± .75	17.5 ± 1.6	18.8 ± 2.0	19.5 ± 2.2	20.0 ± 2.2	20.7 ± 2.8	21.2 ± 3.1°
5	15.3 ± 2.2	19.6 ± 6.1	21.0 ± 6.3	20.2 ± 6.0	22.3 ± 6.5	24.0 ± 3.7	23.8 ± 4.2°°°
6	16.0 ± 1.7	16.2 ± 1.3	16.8 ± 1.7	17.6 ± 1.9	18.4 ± 2.3	19.2 ± 3.0	18.8 ± 2.7°
Stroke Vol							
1	23.4 ± 2.4	25.6 ± 6.0	23.4 ± 5.6	24.3 ± 6.4	28.2 ± 5.9	26.4 ± 13.5	25.5 ± 9.5
2	30.7 ± 6.5	22.3 ± 7.2	21.6 ± 5.7	20.2 ± 6.1	17.2 ± 5.7I	16.5 ± 4.1	18.0 ± 4.4°°
3	32.4 ± 6.6	30.9 ± 6.9	26.3 ± 6.6	25.0 ± 5.5	24.9 ± 4.7*	27.2 ± 4.7*	29.9 ± 3.9
4	27.3 ± 8.1	21.6 ± 1.9	20.9 ± 2.3	18.2 ± 2.0	17.2 ± 2.9	17.9 ± 4.4	19.7 ± 7.2
5	27.7 ± 7.4	27.6 ± 5.3	24.3 ± 4.4	22.1 ± 2.6	22.4 ± 2.7*	23.1 ± 4.1*	24.8 ± 7.7
6	25.6 ± 6.1	29.5 ± 4.8	25.2 ± 5.7	26.4 ± 4.7	24.7 ± 4.0	25.4 ± 4.3	27.5 ± 3.1

Table 5c

Group	Control	0.5 post	1 post	2 post	3 post	4 post	5 post
$Pa0_2$							
1	83.5 ± 4.3	85.0 ± 3.6	85.8 ± 4.2	81.5 ± 4.9	87.0 ± 3.6	82.6 ± 6.2	82.7 ± 7.2
2	82.0 ± 3.7	73.0 ± 7.7	69.3 ± 13.8	68.5 ± 13.9	65.7 ± 13.5^{I}	61.8 ± 17.7^{I}	59.3 ± 16.9$^{I\cdot}$
3	80.8 ± 6.3	71.5 ± 9.6	68.7 ± 9.2	59.7 ± 11.0	61.8 ± 14.8	61.8 ± 12.8	64.3 ± 12.5$^{\cdot}$
4	80.3 ± 7.9	69.5 ± 9.9	66.1 ± 8.3	60.0 ± 5.7	58.0 ± 6.9	58.7 ± 7.2	56.3 ± 7.8$^{\cdot\cdot\cdot}$
5	81.2 ± 2.8	59.3 ± 14.9	60.7 ± 13.3	53.8 ± 12.0	52.3 ± 12.4	50.4 ± 7.7	52.3 ± 12.4$^{\cdot\cdot\cdot}$
6	75.0 ± 5.8	77.8 ± 3.2	78.4 ± 5.3	81.0 ± 5.4	80.2 ± 8.3	82.4 ± 8.3	83.6 ± 7.7$^{\cdot\cdot}$
$Pv0_2$							
1	44.7 ± 1.9	46.0 ± 1.6	46.8 ± 4.5	46.7 ± 4.3	48.0 ± 2.9	44.0 ± 4.6	43.8 ± 7.6
2	45.0 ± 4.2	42.8 ± 4.5	41.0 ± 5.5	39.7 ± 5.2†	38.0 ± 6.1^{I}	35.3 ± 7.9	34.8 ± 8.6$^{\cdot\cdot\cdot}$
3	45.0 ± 5.3	43.8 ± 4.3	43.0 ± 4.4	39.0 ± 6.7	39.3 ± 7.1	41.0 ± 6.2	41.0 ± 5.0$^{\cdot}$
4	43.7 ± 3.9	43.2 ± 3.1	41.3 ± 4.0	36.2 ± 3.1	36.5 ± 3.7	37.0 ± 3.5	34.7 ± 4.0$^{\cdot\cdot\cdot}$
5	44.5 ± 1.4	41.0 ± 7.1	42.5 ± 5.9	37.2 ± 8.4	36.0 ± 7.1	37.0 ± 4.3	36.3 ± 6.6$^{\cdot}$
6	46.2 ± 2.5	47.8 ± 2.7	48.0 ± 1.4	45.0 ± 4.6	44.6 ± 4.3	44.0 ± 3.3	47.4 ± 3.4
$A\text{-}aD0_2$							
1	9.9 ± 2.8	8.6 ± 1.0	7.9 ± 3.4	11.8 ± 1.1	13.4 ± 1.0	13.7 ± 13.1	13.2 ± 3.9
2	11.7 ± 5.2	20.7 ± 7.0^{I}	23.8 ± 8.2^{I}	29.2 ± 12.6^{I}	29.9 ± 13.6	30.1 ± 15.8	30.4 ± 15.9$^{\cdot I}$
3	12.1 ± 4.9	20.8 ± 12.3	25.6 ± 12.7	35.4 ± 13.6	33.4 ± 16.6	36.3 ± 15.6	31.9 ± 15.1$^{\cdot\cdot}$
4	15.6 ± 7.4	26.6 ± 10.2	33.7 ± 10.1	37.8 ± 7.5	38.5 ± 8.5	37.9 ± 8.0	37.9 ± 8.0$^{\cdot\cdot\cdot}$
5	13.9 ± 4.1	27.9 ± 12.6	26.3 ± 10.6	28.2 ± 8.7	31.4 ± 14.5	33.6 ± 7.5	36.2 ± 10.4$^{\cdot\cdot\cdot}$
6	19.3 ± 6.8	16.2 ± 5.1	16.6 ± 3.8	14.3 ± 4.8	14.8 ± 7.8	16.1 ± 7.7	13.7 ± 6.6
Sat A							
1	93.0 ± 1.4	93.5 ± 1.2	93.5 ± 0.8	92.5 ± 1.8	94.0 ± 1.1	92.6 ± 2.8	91.9 ± 4.0
2	92.6 ± 1.2	89.8 ± 3.0	87.2 ± 8.2	87.9 ± 6.9	86.4 ± 7.1	82.0 ± 15.1	80.2 ± 14.2$^{\cdot}$
3	92.4 ± 1.9	88.2 ± 4.7	88.3 ± 3.6	83.2 ± 8.4	82.7 ± 13.1	84.3 ± 8.3	86.0 ± 6.6
4	92.8 ± 1.7	89.4 ± 3.2	88.7 ± 2.8	85.7 ± 3.2	84.5 ± 3.8	84.1 ± 5.4	81.3 ± 8.4$^{\cdot}$
5	93.4 ± 0.8	80.4 ± 13.9	82.2 ± 10.7	73.5 ± 13.3	72.6 ± 13.4	73.1 ± 9.2	72.1 ± 13.3$^{\cdot\cdot}$
6	91.6 ± 1.9	92.2 ± 0.9	92.5 ± 1.5	92.8 ± 1.7	92.5 ± 3.0	93.3 ± 2.1	93.6 ± 1.8

Table 5d

Group	Control	0.5 post	1 post	2 post	3 post	4 post	5 post
Sat V							
1	72.2 ± 3.6	74.8 ± 3.6	74.3 ± 5.6	74.5 ± 4.4	77.4 ± 33.7	71.2 ± 10.3	68.8 ± 14.9
2	72.4 ± 6.0	69.6 ± 7.8	67.4 ± 10.1	65.9 ± 8.3	62.2 ± 8.5^{I}	57.3 ± 16.9	56.3 ± 18.0$^{\cdot}$
3	74.2 ± 2.7	69.4 ± 4.1	69.0 ± 4.1	63.6 ± 9.8	64.4 ± 12.7	66.7 ± 8.4	67.0 ± 6.3
4	72.8 ± 3.9	70.9 ± 3.0	70.0 ± 5.5	62.0 ± 6.1	62.0 ± 7.3	61.8 ± 6.9	57.6 ± 12.9$^{\cdot}$
5	75.1 ± 1.8	65.0 ± 13.1	67.1 ± 10.3	54.0 ± 16.6	53.4 ± 14.5	56.2 ± 10.0	54.6 ± 13.4$^{\cdot\cdot}$
6	75.6 ± 1.0	77.3 ± 2.1	78.1 ± 1.6	74.2 ± 4.8	74.1 ± 5.6	74.7 ± 3.7	77.5 ± 2.7
A-VO_2							
1	3.64 ± 0.64	3.39 ± 0.69	3.45 ± 1.04	3.34 ± 0.70	2.89 ± 0.58	3.92 ± 1.63	4.46 ± 2.21
2	3.31 ± 0.67	3.48 ± 0.33	3.61 ± 0.84	4.18 ± 1.01	4.66 ± 0.87	4.87 ± 1.10	4.32 ± 0.64$^{\cdot\cdot\cdot}$
3	3.36 ± 0.46	3.58 ± 0.89	3.58 ± 0.37	3.70 ± 0.64	3.51 ± 0.29	3.37 ± 0.38*	3.57 ± 0.53
4	3.37 ± 0.64	3.21 ± 0.52	3.29 ± 0.80	4.30 ± 0.86	4.20 ± 1.15	4.23 ± 1.03	4.32 ± 0.87
6	2.94 ± 0.40	2.72 ± 0.39	2.64 ± 0.35	3.41 ± 1.03	3.35 ± 0.96	3.36 ± 0.68	2.92 ± 0.75
$\dot{Q}_S/\dot{Q}_T$							
1	7.4 ± 2.7	6.7 ± 1.6	6.3 ± 3.0	10.5 ± 3.1	9.6 ± 1.2	9.4 ± 2.5	9.4 ± 3.2
2	9.8 ± 2.9	18.7 ± 5.9^{I}	18.4 ± 7.2^{I}	18.2 ± 6.8^{I}	20.9 ± 9.8	20.8 ± 8.8^{I}	25.9 ± 11.7^{I}
3	10.1 ± 4.7	21.0 ± 13.6	23.4 ± 10.0	33.3 ± 13.4*	37.4 ± 18.8	34.4 ± 16.3*	29.2 ± 15.5$^{\cdot}$
4	12.1 ± 6.3	23.5 ± 11.9	27.1 ± 9.7	29.2 ± 6.4*	32.9 ± 10.4	32.2 ± 10.0*	33.5 ± 9.5$^{\cdot\cdot\cdot}$
5	10.8 ± 2.7	36.6 ± 19.2	36.3 ± 17.6	41.8 ± 14.1*	42.8 ± 16.2	48.6 ± 11.9*	49.7 ± 20.8$^{\cdot\cdot\cdot}$
6	18.4 ± 8.1†	16.1 ± 4.8	16.3 ± 4.6†	11.1 ± 3.5	12.0 ± 7.6	11.3 ± 6.5	11.4 ± 7.6$^{\cdot}$
$\dot{V}O_2$							
1	141.4 ± 29.3	169.1 ± 67.0	160.1 ± 76.1	131.4 ± 50.3	148.9 ± 64.3	141.5 ± 60.5	164.7 ± 69.4
2	173.8 ± 62.8	150.4 ± 37.6	158.3 ± 64.6	153.4 ± 66.1	148.6 ± 40.5	155.4 ± 43.9	143.3 ± 32.0
3	154.6 ± 21.7	162.7 ± 41.1	149.9 ± 20.0	147.6 ± 22.9	143.8 ± 23.5	154.9 ± 21.2	179.3 ± 21.8
4	148.8 ± 28.1	115.9 ± 18.6*	114.4 ± 24.7	134.6 ± 32.3	126.5 ± 49.3	121.2 ± 33.4	132.5 ± 45.1
5	126.2 ± 29.5	107.7 ± 14.5*	98.3 ± 24.7	147.2 ± 52.8	143.2 ± 34.7	130.2 ± 26.9	127.1 ± 51.7
6	114.0 ± 27.9	105.7 ± 11.5	106.0 ± 20.5	135.1 ± 37.1	123.7 ± 37.1	130.6 ± 42.3	124.6 ± 26.3

Table 5e

Group	Control	0.5 post	1 post	2 post	3 post	4 post	5 post
Arterial Platelets							
1	184.2 ± 21.9	161.0 ± 6.1	-	-	-	-	171.7 ± 17.1
2	177.8 ± 24.6	184.3 ± 2.9[I]	-	-	-	-	178.2 ± 40.1
3	217.8 ± 36.7	226.6 ± 30.9	-	-	-	-	214.7 ± 34.4
4	199.0 ± 66.6	205.8 ± 69.4	-	-	-	-	201.2 ± 57.4
5	188.0 ± 43.7	190.2 ± 47.5	-	-	-	-	205.6 ± 61.7
6	197.4 ± 22.8	203.2 ± 31.4	-	-	-	-	210.6 ± 22.2
Venous Platelets							
1	167.2 ± 23.2	164.3 ± 11.6	-	-	-	-	159.0 ± 11.0
2	162.0 ± 25.9	189.7 ± 11.4	-	-	-	-	195.0 ± 46.8
3	210.0 ± 35.2	227.5 ± 44.1	-	-	-	-	220.3 ± 30.5
4	186.7 ± 61.2	211.7 ± 53.6	-	-	-	-	196.2 ± 67.2
5	176.5 ± 28.3	218.8 ± 60.8	-	-	-	-	218.4 ± 53.4
6	185.8 ± 14.2	204.2 ± 29.1	-	-	-	-	215.6 ± 26.0
Indium^{111} Count Rate - arterial expressed as % of control measurement							
1	0	-.044 ± .120	-.090 ± .171	-.150 ± 0	-	-	-.142 ± .192
2	0	-.247 ± .059[I]	-.144 ± .113	-.204 ± .068	-	-	-.160 ± .065**
3	0	-.161 ± .143	-.217 ± .060	-.279 ± 0	-	-	-.316 ± .058***
4	0	-.094 ± .122	-.105 ± .105	-.092 ± .094	-	-	-.108 ± .113
5	0	-.154 ± .121	-.223 ± .151	-.254 ± .139	-	-	-.263 ± .121***
6	0	-.196 ± .076[†]	-.247 ± .073[†]	-.248 ± .079	-	-	-.241 ± .067***
Indium^{111} Count Rate - venous expressed as % of control measurement							
1	0	-.025 ± .115	-.263 ± .037	-.014 ± 0	-	-	-.071 ± .170
2	0	-.028 ± .118	-.160 ± .032	-.235 ± .069	-	-	-.140 ± .138
3	0	-.158 ± .106	-.216 ± 0	-.189 ± 0	-	-	-.013 ± .142
4	0	-.088 ± .137	-.105 ± .146	-.073 ± .215	-	-	-.110 ± .154
5	0	-.160 ± .115	-.196 ± .146	-.238 ± .140	-	-	-.165 ± .158
6	0	-.268 ± .091[†]	-.309 ± .101	-.310 ± .097	-	-	-.276 ± .070***

Table 5f

Group

Radiation Lung - corrected by dividing blood radiation

1	.482 ± .158
2	.779 ± .089^{II}
3	.766 ± .146
4	.831 ± .172
5	.736 ± .203
6	.482 ± .139

Wet/Dry Ratio

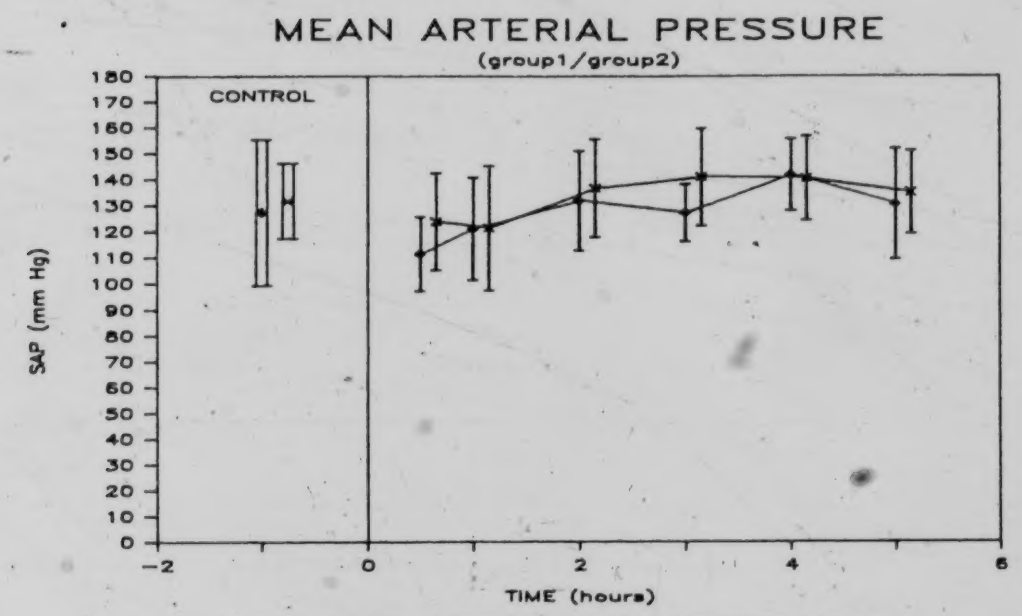
1	4.56 ± 0.84
2	7.77 ± 0.57^{II}
3	8.35 ± 0.74
4	7.70 ± 0.75
5	8.53 ± 0.41
6	5.31 ± 0.44

Residual blood volume of lung

1	0.35 ± 0.08
2	0.25 ± 0.07*
3	0.18 ± 0.02*
4	0.26 ± 0.05*
5	0.21 ± 0.03*
6	0.27 ± 0.03

*	p < 0.05	Groups 3,4 or 5 compared to Group 2	ANOVA
**	p < 0.01	Groups 3,4 or 5 compared to Group 2	ANOVA
I	p < 0.05	Group 2 compared to Group 1	ANOVA
II	p < 0.01	Group 2 compared to Group 1	ANOVA
†	p < 0.05	Group 6 compared to Group 1	ANOVA
††	p < 0.01	Group 6 compared to Group 1	ANOVA
·	p < 0.05	5 hour post on value compared to control value	paired t-test
··	p < 0.02	5 hour post on value compared to control value	paired t-test
···	p < 0.01	5 hour post on value compared to control value	paired t-test

Figure 3 - Mean Arterial Pressure Versus Time
Time 0 hours corresponds to the right ventricular injection of saline or oleic acid.
Group 1: saline controls;
Group 2: oleic acid controls;
Group 6: saline and prostacyclin infusion
◆ Group 1 (n = 6)
X Group 2 (n = 6)
X Group 6 (n = 5)



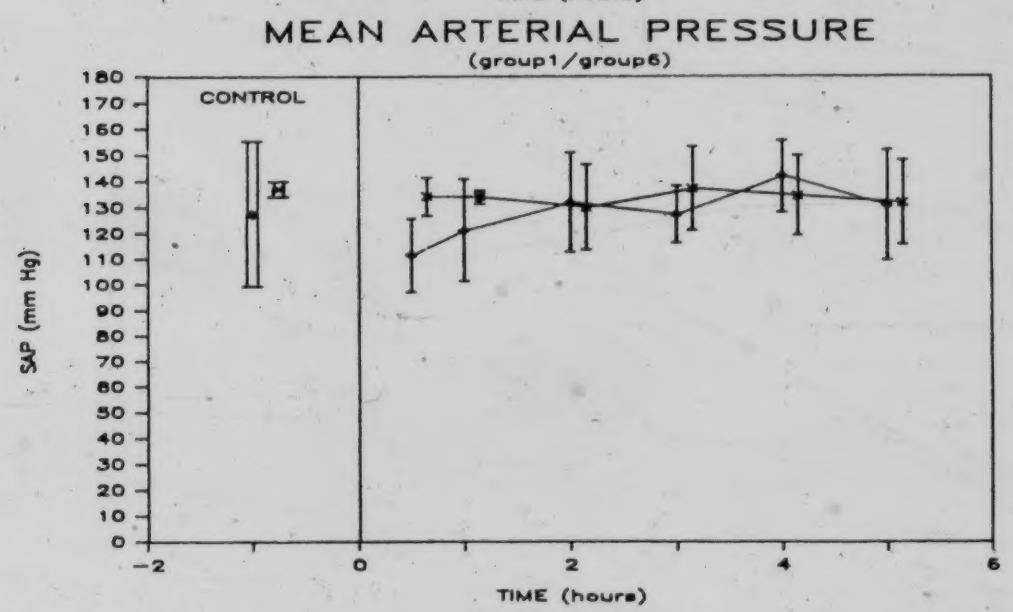
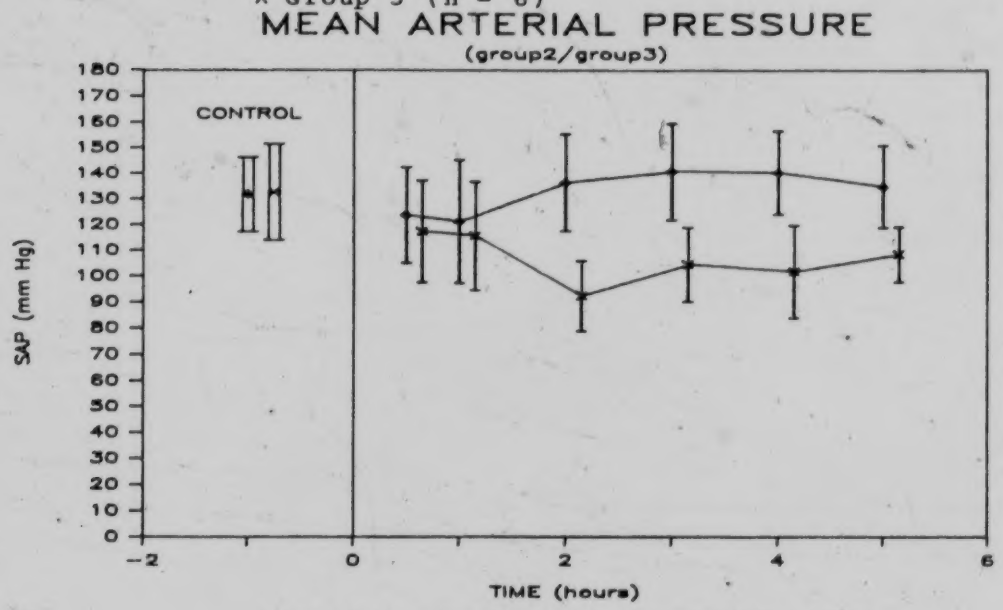


Figure 4 - Mean Arterial Pressure Versus Time
Time 0 hours corresponds to the right ventricular injection of oleic acid.
Infusions of drugs commenced at +1 hour.
Group 2: oleic acid controls;
Group 3: oleic acid and prostacyclin;
Group 4: oleic acid and aminophylline;
Group 5: oleic acid, prostacyclin and aminophylline

◆ Group 2 (n = 6)
X Group 3 (n = 6)
◆ Group 4 (n = 6)
X Group 5 (n = 6)



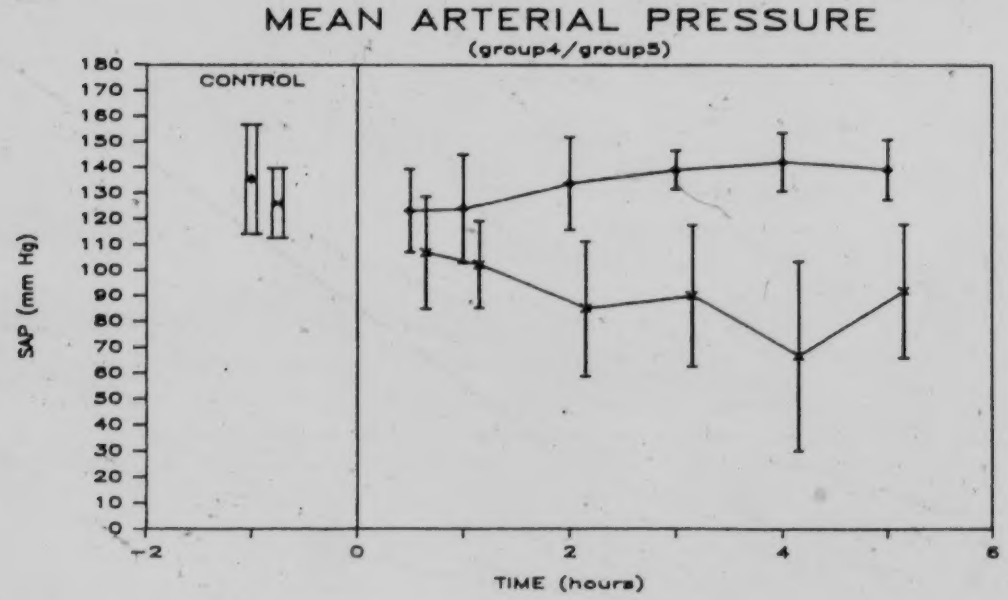
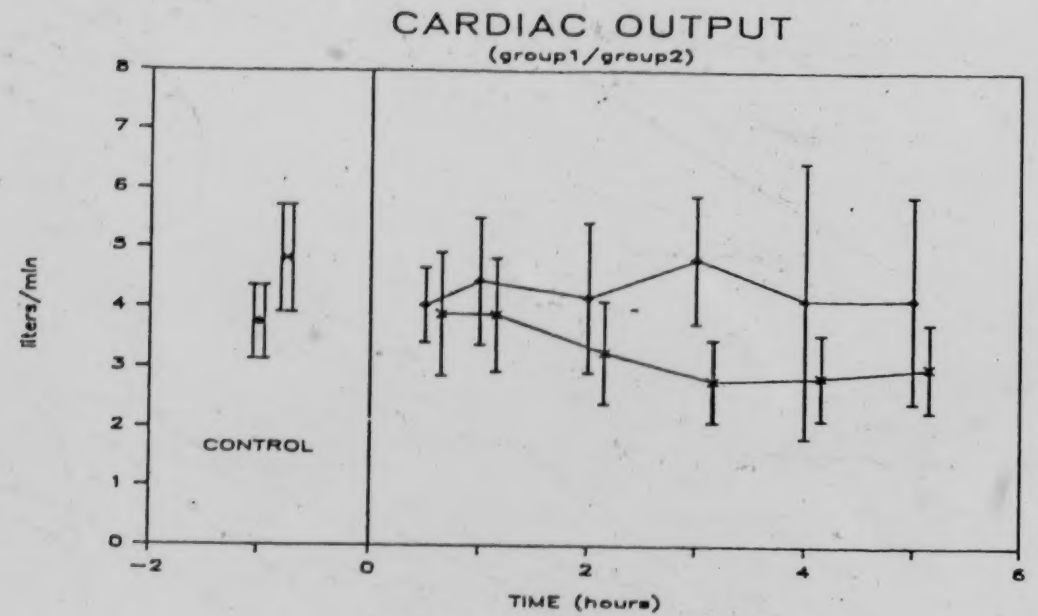


Figure 5 - Cardiac Output Versus Time
Time 0 hours corresponds to the right ventricular injection of saline of oleic acid.
Group 1: saline controls;
Group 2: oleic acid controls;
Group 6: saline and prostacyclin infusion
◆ Group 1 (n = 6)
X Group 2 (n = 6)
X Group 6 (n = 5)



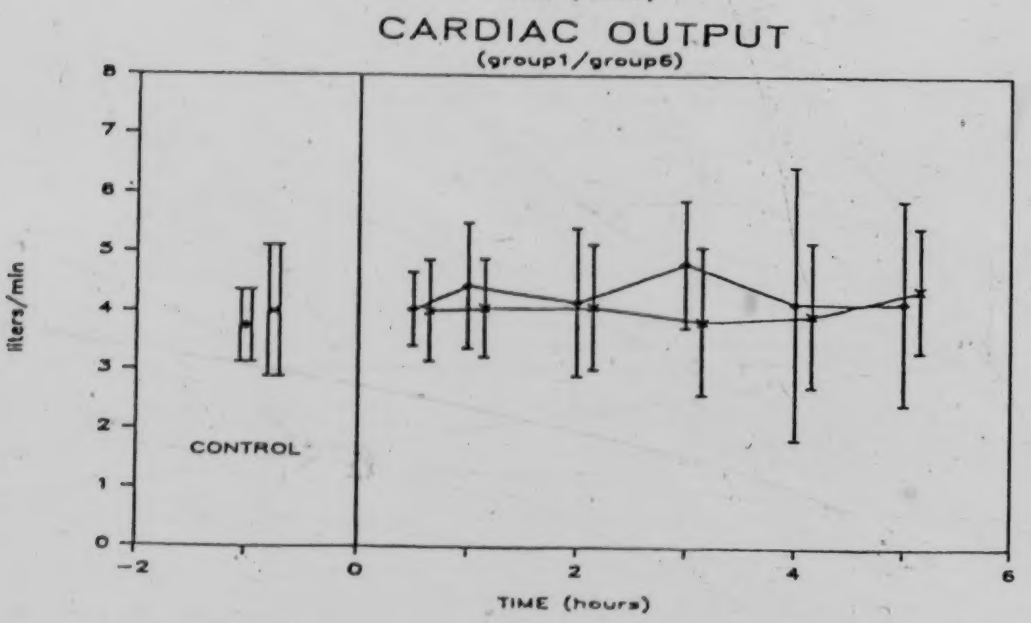
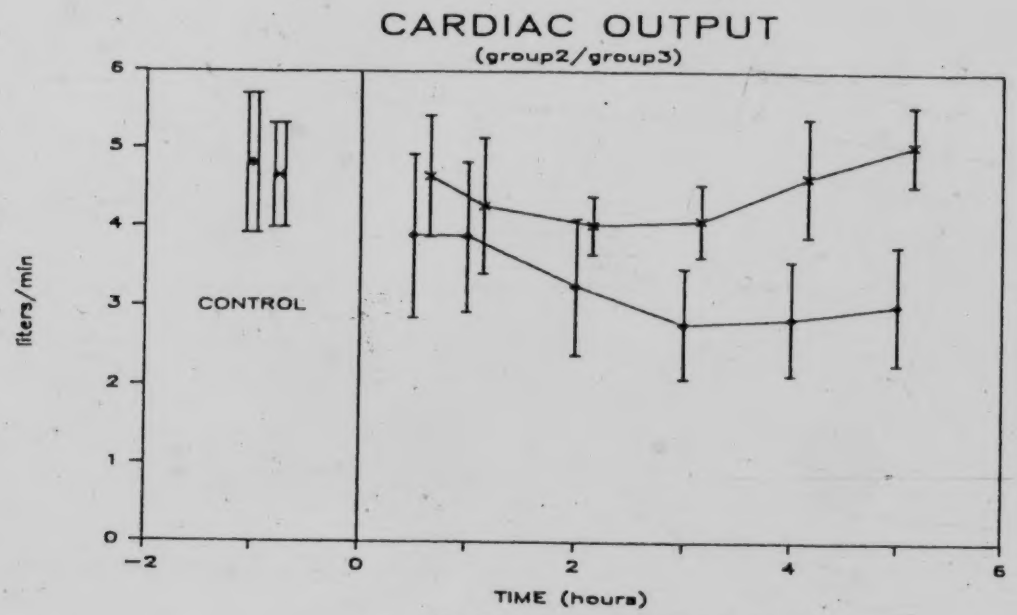


Figure 6 - Cardiac Output Versus Time
Time 0 hours corresponds to the right ventricular injection of oleic acid.
Infusions of drugs commenced at +1 hour.
Group 2: oleic acid controls;
Group 3: oleic acid and prostacyclin;
Group 4: oleic acid and aminophylline;
Group 5: oleic acid, prostacyclin and aminophylline

◆ Group 2 (n = 6)
X Group 3 (n = 6)
◆ Group 4 (n = 6)
X Group 5 (n = 6)



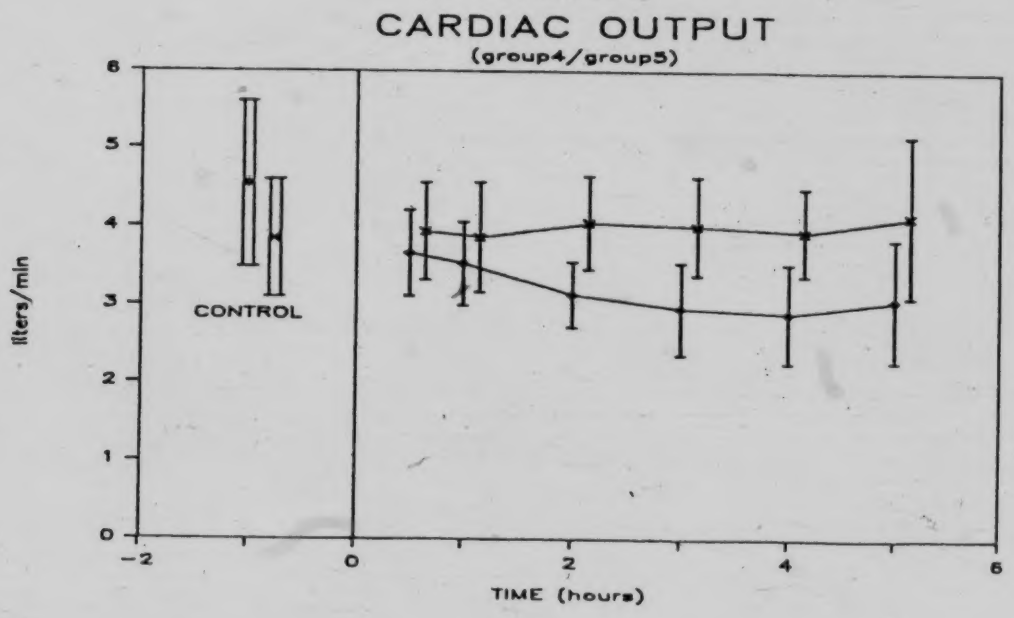
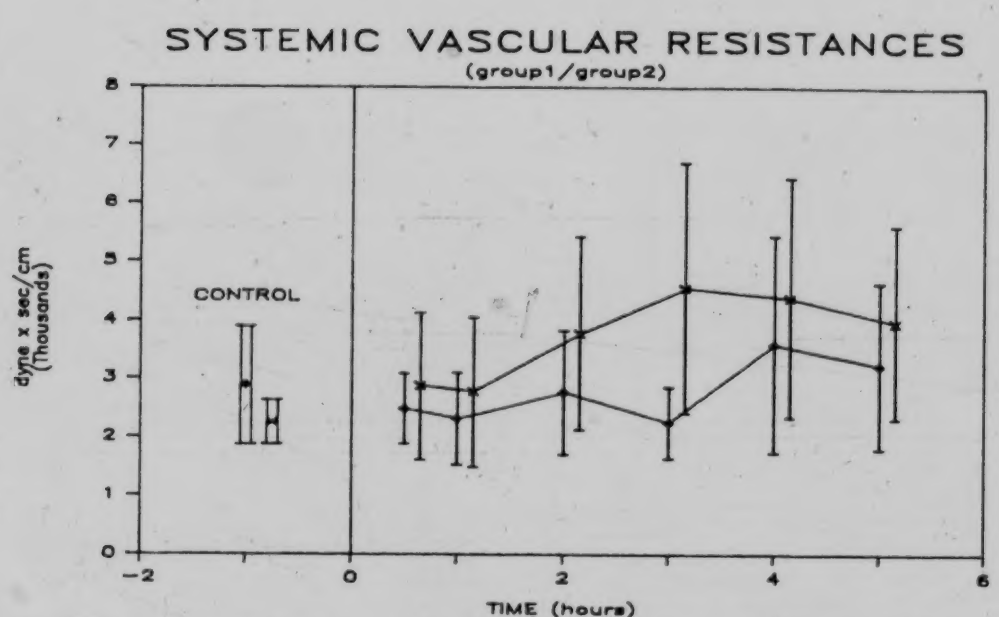


Figure 7 - Systemic Vascular Resistance
Time 0 hours corresponds to the right ventricular injection of saline or oleic acid.
Group 1: saline controls;
Group 2: oleic acid controls;
Group 6: saline and prostacyclin infusion
◆ Group 1 (n = 6)
X Group 2 (n = 6)
X Group 6 (n = 5)



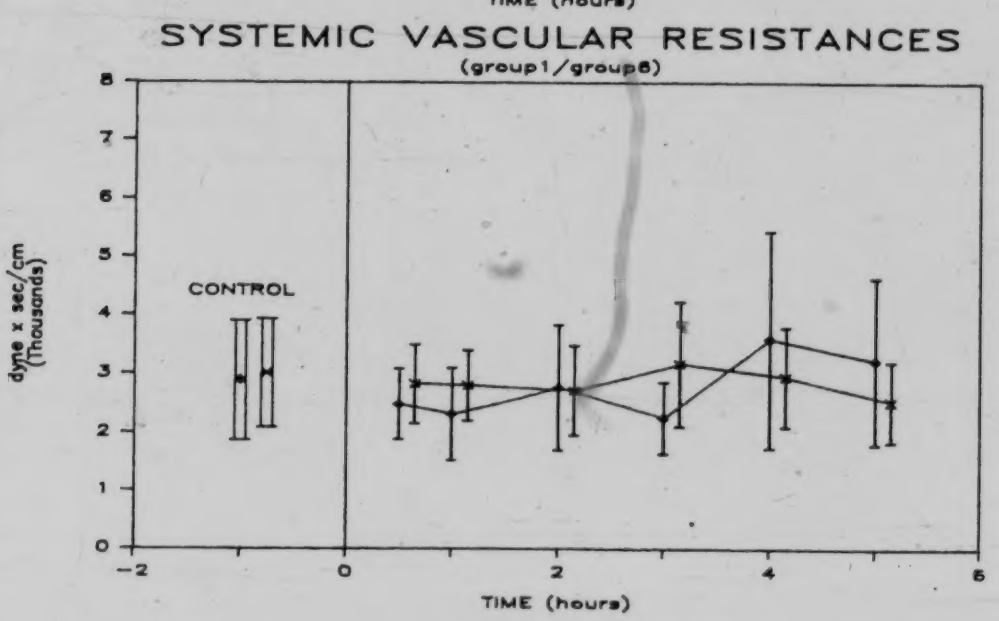
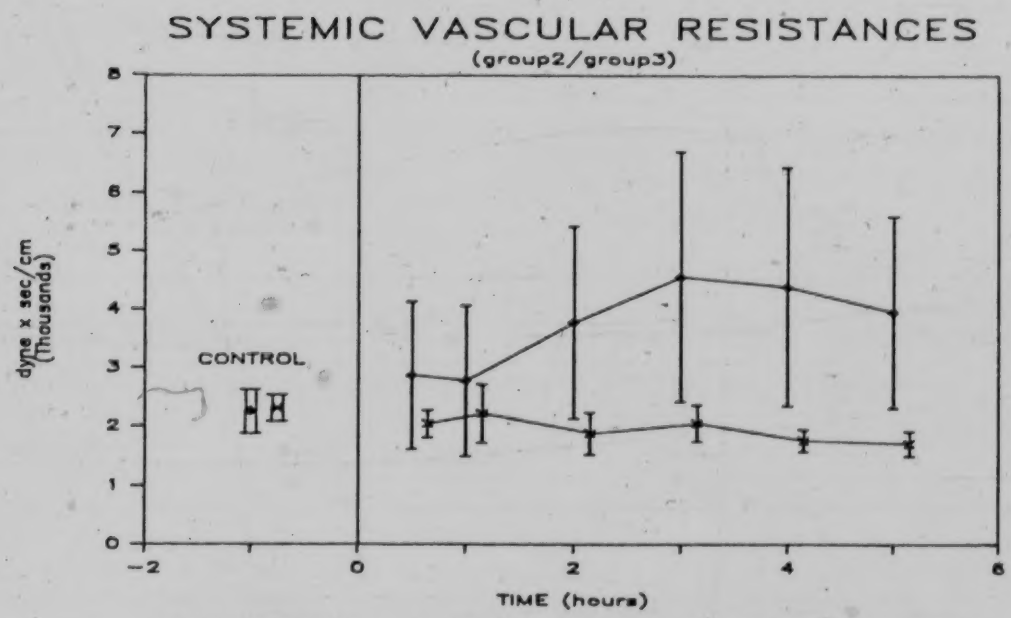
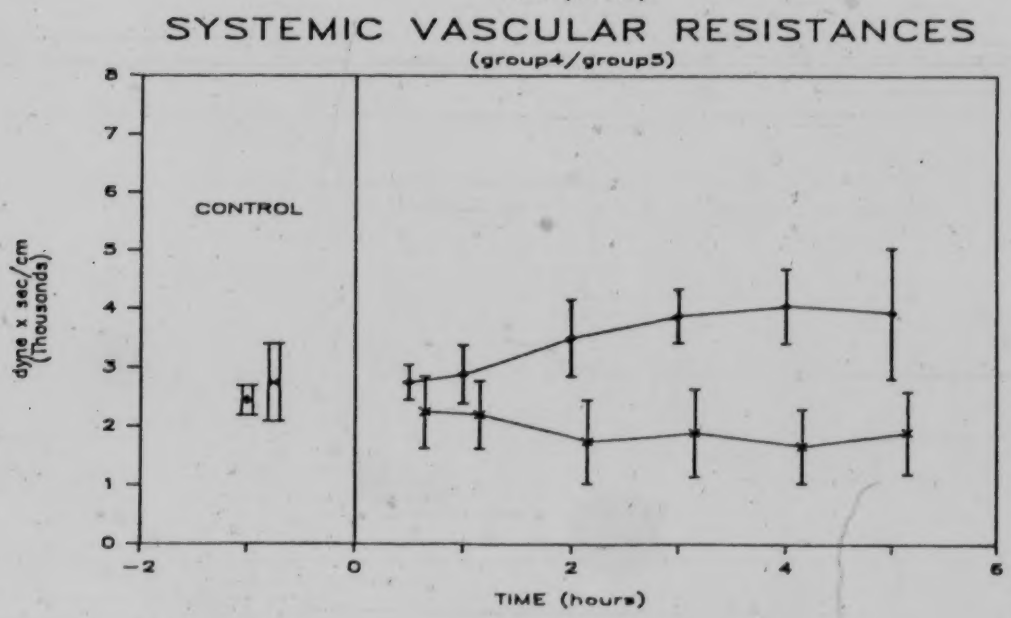


Figure 8 - Systemic Vascular Resistance
Time 0 corresponds to the right ventricular injection of oleic acid.
Infusions of drugs commenced at +1 hour.
◆ Group 2: oleic acid controls;
X Group 3: oleic acid and prostacyclin;
◆ Group 4: oleic acid and aminophylline;
X Group 5: oleic acid, prostacyclin and aminophylline
Group 2 (n = 6)
Group 3 (n = 6)
Group 4 (n = 6)
Group 5 (n = 6)





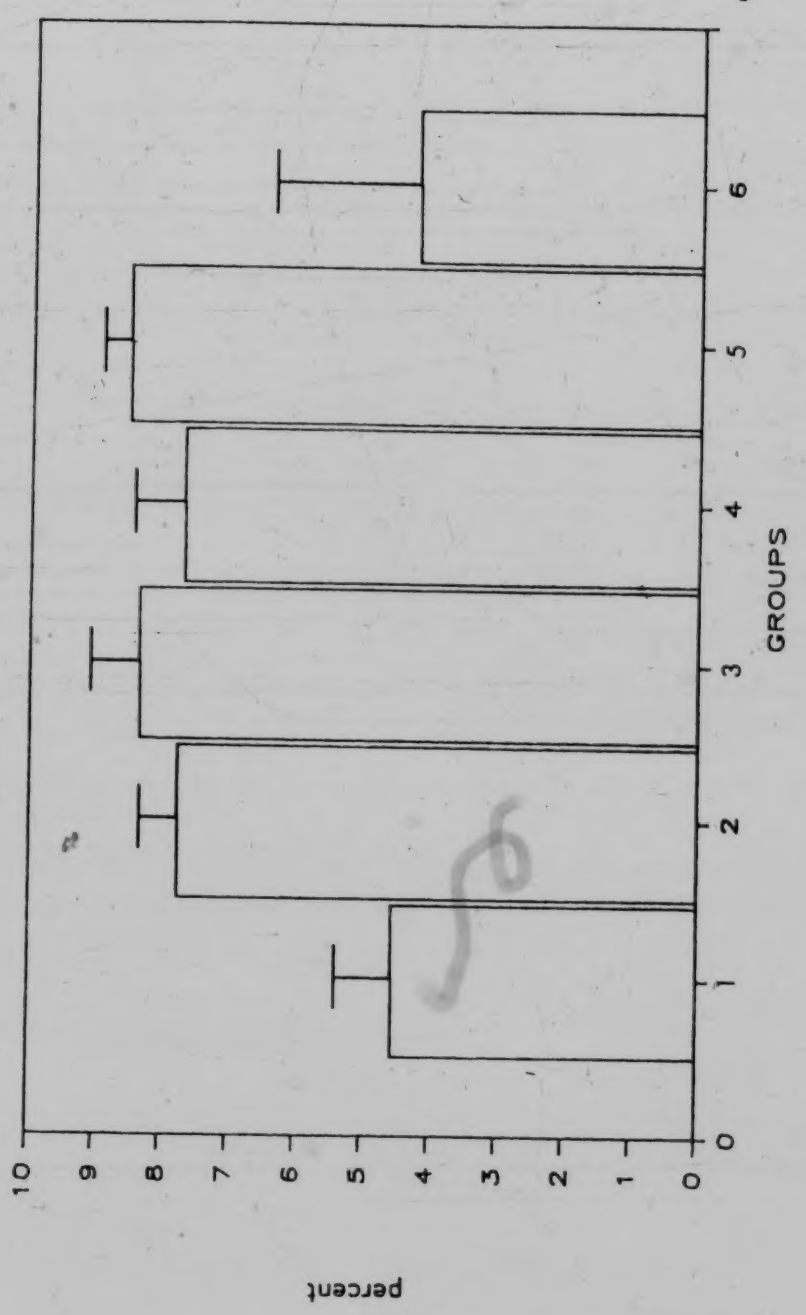


Figure 9 - Lung Wet/Dry Ratio
Groups 1-5 (n = 6); Group 6 (n = 5)

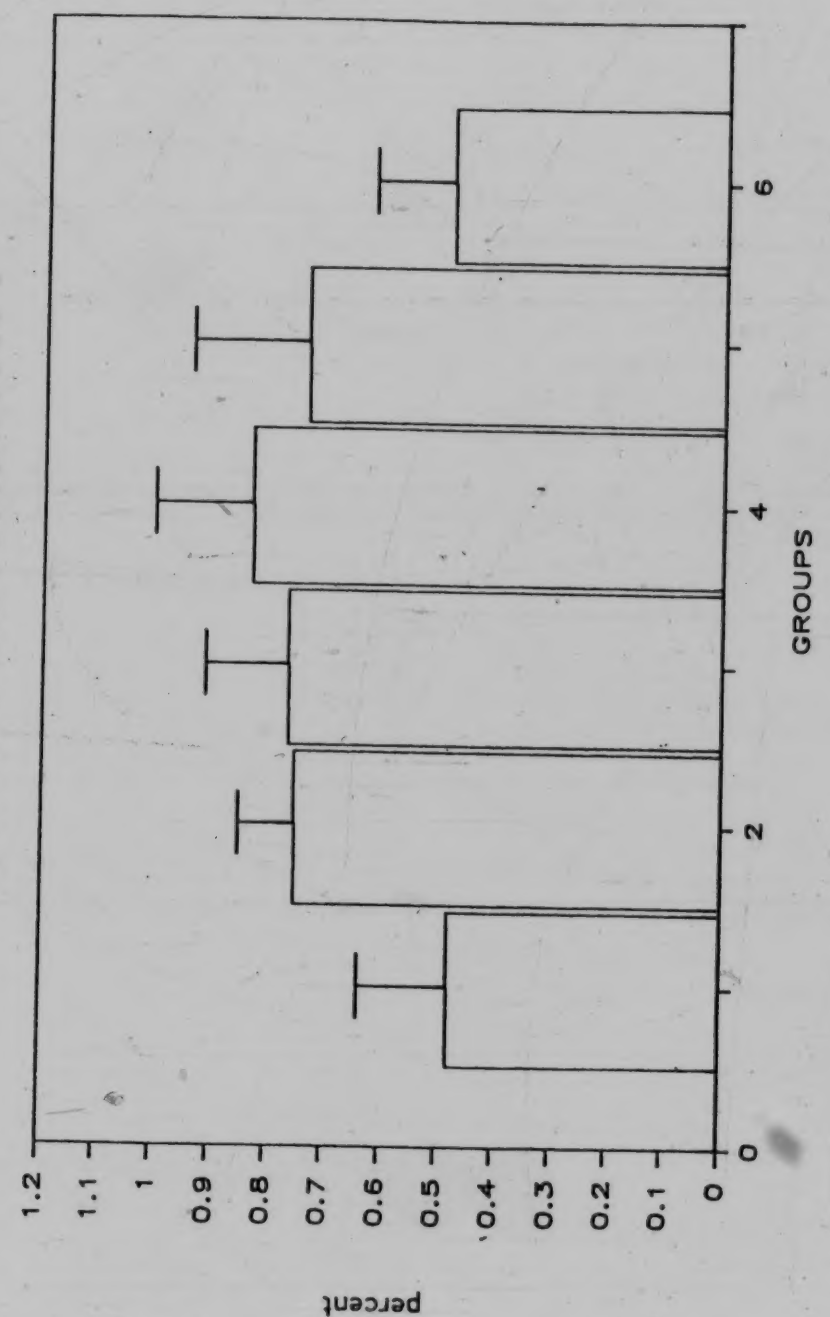
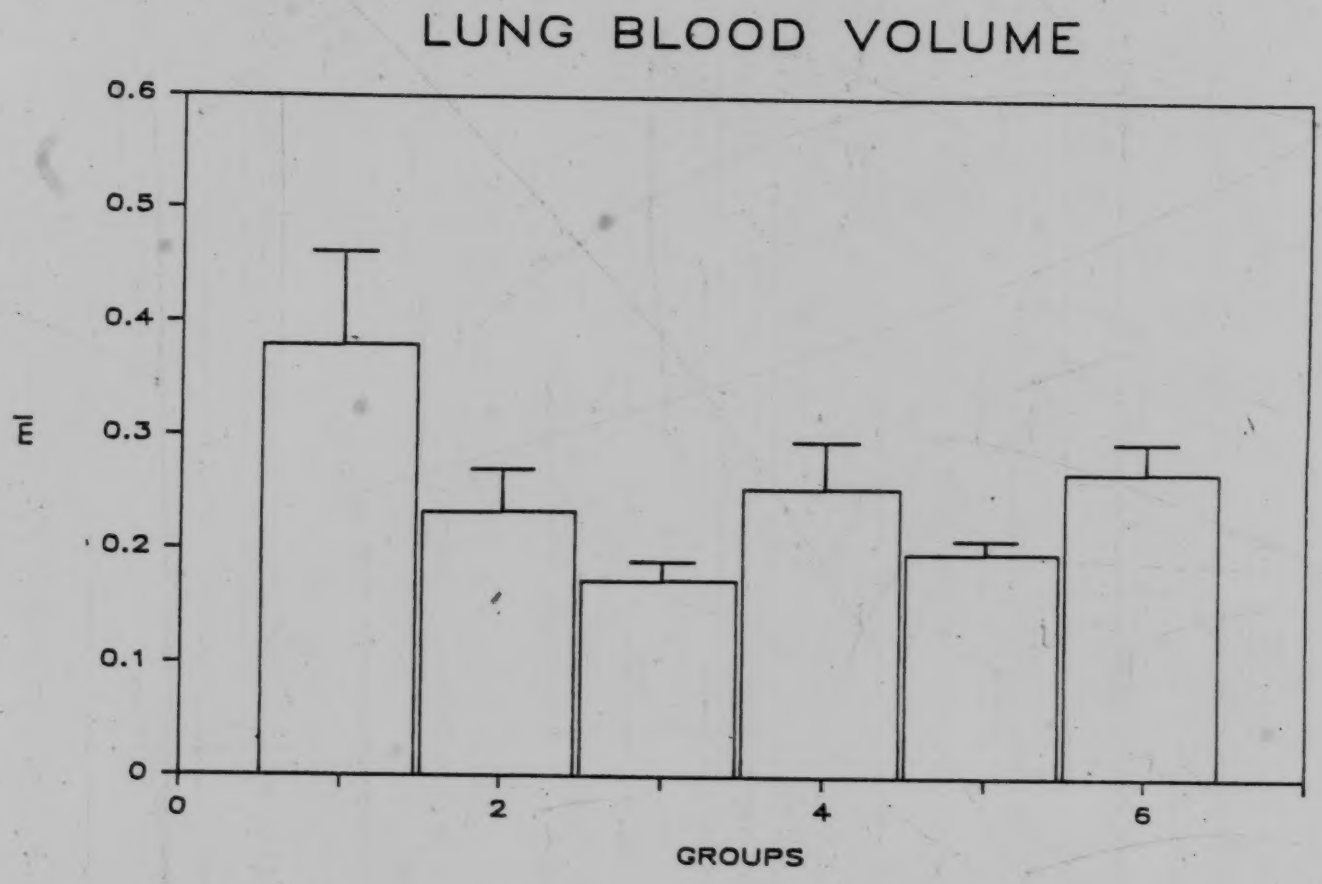


Figure 10 - Lung Radioactivity
Groups 1-5 (n = 6); Group 6 (n = 5)

Figure 11 - Lung Blood Volume
Groups 1-5 (n = 6); Group 6 (n = 5)



DISCUSSION

Platelets have been implicated by others as the primary etiologic agent in the adult respiratory distress syndrome (Connell et al, 1975, Blaisdell et al, 1970). Stolman and coworkers (1982) suggest that prostacyclin diminished oleic acid injury and postulated that this effect was due to platelet aggregation inhibition.

The dose of prostacyclin (100ng/kg/min) was chosen to minimize the cardiovascular insult while at the same time maximizing the platelet inhibition effect. The assumption that inhibition of pulmonary platelet aggregation plays a role in reducing lung damage induced by oleic acid has been made by some authors (Slotman et al, 1982, Miyazawa et al, 1982). Slotman and his group (1982) infused prostacyclin at a dose of 300ng/kg/min into dogs with oleic acid induced pulmonary injury. They demonstrated a 57% reduction in MAP. This degree of cardiovascular instability makes this dose of drug unsuitable in most clinical situations. Miyazawa and coworkers (1982) did not report the hemodynamic effects of prostacyclin in their study. In order to avoid hemodynamic instability, a lower dose of prostacyclin (100ng/kg/min) was selected. As already mentioned, this dose retains maximal in vivo platelet aggregation inhibition as measured by bleeding time (Ubatuba et al, 1979).

Demling et al (1981) were forced to titrate the dose of prostacyclin between 100 and 200ng/kg/min in the endotoxin-induced lung injury model because of vasodilation.

Using a dose of 100ng/kg/min, a 26% reduction in MAP took place in dogs receiving oleic acid and prostacyclin infusion. When prostacyclin was combined with aminophylline, a 37% reduction in MAP took place. While the difference in MAP between the group recievng oleic acid and prostacyclin (Group 3) and those receiving oleic acid, aminophylline and prostacyclin (Group 5) were not statistically significant, a drug interaction may have taken place resulting in the potentiation of prostacyclin by aminophylline. Patients with multisystemic disorders will not tolerate 30% reductions in MAP. It is apparent that prostacyclin may have to be used in lower doses in clinical practice which could theoretically result in loss of platelet aggregation inhibition.

It is difficult to rationalize the lack of change of MAP in the group receiving saline and prostacyclin (Group 6). Prewitt and Wood (1981) demonstrated that sodium nitroprusside administered to healthy anesthetized dogs resulted in a fall of blood pressure, stroke volume and pulmonary capillary wedge pressure. This caused a compensatory tachycardia with an unchanged cardiac

output. With the exception of a mild reduction in MAP which was not statistically significant, none of these events occurred in our healthy anesthetized animals given prostacyclin alone (Group 6). This leads to two possibilities. First, the dose administered was subtherapeutic with regard to the cardiovascular parameters in healthy animals without pulmonary injury. That is, injuring the lungs or myocardium with oleic acid renders the animals more susceptable to the pharmacological effect of prostacyclin. This seems unlikely as a significant fall in mean arterial pressure and a rise in heart rate and cardiac output in healthy anesthetized dogs receiving as little as 50ng/kg/min of prostacyclin for three minutes has been reported (Armstrong et al, 1977). The second possibility is perhaps more likely. That is, there may have been a loss of potency through storage of the drug. The refrigeration unit storing the prostacyclin was accidentially turned off just prior to the group 6 trial after experimentation on all other groups had been completed. The time without refrigeration was unknown but estimated to be at least 72 hours during which time the prostacyclin attained room temperature. Some of the drug potency was likely lost even though in vitro platelet testing suggested this loss to be trivial.

Reconstituted prostacyclin in a buffered solution with a pH of 10 will loose approximately 10% of its activity every 6-15 hours at 25°C. It seems possible then that the lack of pharmacological action of prostacyclin on group 6 animals was on the basis of damaged drug. This likely invalidates any conclusions that could be drawn from this group. The group 6 trial was not repeated because it added little information to the study. Secondly, the funding for the study had been exhausted at that point in time.

This study is in agreement with others that have demonstrated a reduction in cardiac output as a result of oleic acid injection (Shuster and Trulock, 1984, Prewitt and Woods, 1981). The cardiac dysfunction appears to take the form of diminished myocardial contractility and is independent of preload considerations. However, the etiology remains unclear and a study looking at left ventricular performance with respect to filling volumes is needed in this model. It is possible that oleic acid is a direct myocardial toxin damaging the right ventricle causing a change in the myocardial compliance curve or the damaged lung releases mediators that impair myocardial contractility. Another possibility is that oleic acid raises systemic vascular resistance by an unknown mechanism which impedes left ventricular ejection

(Prewitt and Wood 1981). This suggestion is unlikely as there was no dramatic rise in MAP. The pattern of a falling cardiac ouput and rising SVR with an unchanged MAP suggesting a myocardial contractility lesion is more likely. Certainly stroke volume fell significantly by 41% over the 5 hour course of our experiment in the dogs given oleic acid alone (Group 2).

Slotman and coworkers (1982) have demonstrated and we now confirm that prostacyclin tends to maintain cardiac output at the preoleic acid levels. Demling et al (1981) demonstrated a similar phenomenon in the endotoxin pulmonary injury model. This ability comes from mild elevation of both stroke volume and heart rate when compared to the oleic acid controls. While no significant difference could be demonstrated between prostacyclin and nonprostacyclin oleic acid injected dogs with respect to heart rate or stroke volume, the trend demonstrates an elevation of both in the prostacyclin infused dogs. The change in heart rate was indeed small but stroke volume appears to be maintained near the pre-oleic acid value in the prostacyclin infused groups (3 and 5). Aminophylline appears to have no demonstratable effect either by itself or as an adjunct to prostacyclin.

SVR mirrors changes already discussed under cardiac output and MAP. Although animals receiving aminophylline

and prostacyclin (Group 5) had a slightly greater reduction of SVR when compared to those receiving prostacyclin (Group 3), the difference was minimal and probably reflected the slightly greater reduction in MAP seen in animals receiving aminophylline and prostacyclin. These findings are in agreement with those of Slotman et al (1982) in the oleic acid-induced lung injury model and Demling et al (1981) in the endotoxin-induced lung injury model.

Prostacyclin blunted the oleic acid induced rise in PAP. This finding is similar to those of Slotman et al (1982) and Demling et al (1981). Miyazawa et al (1982) measured PAP in their experiment of injuring dog lungs with oleic acid followed by prostacyclin infusion, but they did not present any results dealing with PAP. The combination of aminophylline and prostacyclin infusions appeared to be synergistic in nature resulting in a significantly lower PAP when compared to all other groups given oleic acid. This is not surprising as both prostacyclin and aminophylline are well known pulmonary vasodilators operating at different levels of the intracellular chemical messenger system. Prostacyclin is a potent agonist for adenylate cyclase while aminophylline is a phosphodiesterase inhibitor (Tateson et al, 1977) or an adenosine antagonist (Person et al,

1982). This synergistic effect has been confirmed in vitro (Tsien et al, 1982). Closer examination of the PVR revealed a different pattern, in that the rise in PVR induced by oleic acid was blunted in the prostacyclin groups only. Aminophylline by itself (Group 4) appeared to be ineffective in blunting this rise. PVR is a derived value incorporating mean pulmonary artery pressure and cardiac output. Aminophylline by itself was ineffective in significantly raising cardiac output. Therefore its effect on PVR appears to be minimal. These results agree with those of Slotman in that prostacyclin infusion blunts the rise in PVR when compared to animals given oleic acid alone (Slotman et al, 1982). The phenomenon of normalization of PVR after lung injury has also been demonstrated in the endotoxin model (Demling et al, 1981).

It is interesting to note that the combination of aminophylline and prostacyclin produced a synergistic effect only when PAP was examined. However a similar trend, though not statistically significant, occurred with MAP. It would appear that the dose of aminophylline was probably subtherapeutic to inhibit phosphodiesterase. The observed effects of aminophylline were probably then due to adenosine antagonism (Person et al, 1982).

No consistent findings were observed when $Pa0_2$ was examined. Oleic acid reduced $Pa0_2$ in all groups. This is not particularly surprising as two opposing forces are at play. First pulmonary vasodilators increase $\dot{Q}_S/\dot{Q}_T$ in oleic acid induced pulmonary injury. This has been confirmed by Prewitt and Wood (1981) using sodium nitroprusside. A similar pattern can be seen in this study. $\dot{Q}_S/\dot{Q}_T$ was raised in all groups receiving oleic acid; however, $\dot{Q}_S/\dot{Q}_T$ was elevated further in both prostacyclin and aminophylline groups when compared to the group given oleic acid alone. This effect was even greater in the group given both prostacyclin and aminophylline suggesting a degree of synergism. These results were also statistically significant 2 and 4 hours postoleic acid administration for all groups. Prewitt and Wood (1981) postulated several mechanisms to explain the elevation of $\dot{Q}_S/\dot{Q}_T$ with increased cardiac output. These include: 1) vascular recruitment of nonventilated areas; ii) decreased transit time by increasing blood velocity across the alveolus thus augmenting any diffusion defect for oxygen; and, iii) raised cardiac output resulting in increased pulmonary edema by increasing the hydrostatic pressure in the injured areas. A factor opposing the aforementioned effects, is that an elevated cardiac output from vasodilation results

in improved tissue perfusion which would increase mixed venous PO_2 and therefore decrease the effect of venous admixture on arterial PO_2. The $P\bar{v}O_2$ fell in all groups given oleic acid but this fall was less dramatic in those given prostacyclin. However, this trend was small and statistically insignificant. A-VDO_2 also conformed to the aforementioned trend; i.e., A-VDO_2 was smaller in the prostacyclin groups suggesting improved tissue oxygen delivery because of augmented cardiac output. This study agrees with Slotman et al (1982) who also found a reduced A-VDO_2 in oleic acid embolized dogs receiving prostacyclin.

The A-aDO_2 fell in all groups that received oleic acid. No intergroup differences were observed. These results are at odds with Miyazawa et al (1982) who demonstrated a fall in A-aDO_2 with prostacyclin infusion after oleic acid pulmonary injury. Perhaps the difference lies in the fact that P_AO_2 was estimated as end tidal PO_2 ($P_{ET}O_2$) in their study whereas P_AO_2 was calculated from the alveolar air equation in this study. $P_{ET}O_2$ as an estimate of P_AO_2 has one major drawback. The presence of a significant number of underperfused alveoli (dead space) will return gas with a higher P_AO_2. Miyazawa et al in the same experiment demonstrated a rise in V_D/V_T after oleic acid

administration. The V_D/V_T fell with prostacyclin administration in their study thereby lowering P_A0_2. As P_A0_2 was overestimated initially this would be perceived as an apparent improvement, when none had occurred. $Pa0_2$ in their experiment, as in ours, did not improve with prostacyclin infusion. A calculated P_A0_2 from the alveolar air equation would not be influenced by the phenomenon.

Slotman in an earlier study of prostacylin on oleic acid induced lung injury concluded that prostacyclin had a beneficial effect in hypoxic respiratory failure by improving pulmonary gas exchange and tissue oxygen delivery (Slotman et al, 1979). Miyazawa et al (1982) noted decreased respiratory distress and respiratory rate in spontaneously breathing dogs with oleic acid induced pulmonary injury who were infused with prostacyclin. They also suggested improved carbon dioxide clearance as estimated by reduced $\dot{V}_E$ and V_D/V_T in animals given this drug. Our study also indicates that prostacyclin improved tissue perfusion and oxygen availability. Oxygen availability is greater in the prostacyclin-infused animals when compared to the oleic acid control animals (816ml/min vs 449ml/min). This represents almost a two-fold difference. Miyazawa's observations of diminished $\dot{V}_E$ and respiratory rate can be explained by

their measured fall in V_D/V_T induced by prostacyclin infusion (Miyazawa et al, 1982). Another possible explanation for Miyazawa's these results was improved tissue blood flow induced by prostacyclin mediated increased cardiac output resulting in greater oxygen and substrate availability to all organs including the respiratory muscles.

Throughout the five hour course of our experiment, there was no change in either arterial or venous platelet counts. The arterial and venous blood indium^{111} count rate fell in all groups. When count rate in arterial blood was examined more closely, there appeared to be a greater fall in the count rate of the prostacyclin groups (3 and 5). This fall was significant when compared to the nonprostacyclin oleic acid groups (2 and 4). The explanation for this phenomenon is difficult. Perhaps improved cardiac output in the prostacyclin groups allowed more platelets to be presented to the oleic acid damaged endothelial membranes. While the fall in arterial whole blood indium^{111} count rate was significant in most oleic acid groups when compared to their control values, the change in venous whole blood radioactivity was minimal with all groups including controls having a similar magnitude of reduction. This reduction was not statistically significant. These results suggest two conclusions.

Certainly some indium^{111} labelled platelets were lost over the duration of the experiment as demonstrated by the control animals. However, some platelets were sequestered in the injured lung. Confirming this fact is the marked increase in lung count rate on all oleic acid groups. These sequestered platelets must have been rapidly replaced from a bone marrow stores in order to maintain the platelet count. We were unable to demonstrate an arterial-venous gradient in platelet counts. This observation is in agreement with Hechtman et al (1978b) who could not demonstrate an arterial-venous platelet gradient in septic patients with hypoxemic respiratory failure. Another possible source of error here is the collection of venous platelets through a 100cm polyethylene pulmonary artery catheter. Theoretically platelets might stick to the catheter wall, thus artifically lowering the venous platelet count. A heparin-bonded pulmonary artery catheter could help avoid this problem. The various different treatment modalities made no difference on uptake of radioactive platelets by the lung.

Platelets were implicated as an etiologic agent of hypoxemic respiratory failure in the early 1970's when ultrastructural studies demonstrated platelet microaggregates in dog lungs injured by hypovolemic shock

(Connell et al, 1975) and at autopsy in humans dying of traumatic shock (Blaisdell et al, 1970). Disseminated intravascular coagulation was suggested as the triggering event to pulmonary injury. Bridenberg et al (1977, 1980) demonstrated that the phase I response in endotoxin-induced pulmonary injury could be prevented by rendering their test animals thrombocytopenic by antiplatelet serum. Further studies have questioned this relationship. First of all, attempts at producing a valid model of lung injury induced by disseminated intravascular coagulation have been unsuccessful (Vaage 1982). Myrvold (1976) demonstrated in thrombocytopenic dogs, unaltered pulmonary damage and leukocyte sequestration in the endotoxin injury model. Hechtman et al (1978a) utilizing a double indicator technique, demonstrated pulmonary platelet sequestration in both oleic acid and endotoxin pulmonary injury models. Platelet sequestration was independent of the model used. They also found no correlation with measures of pulmonary damage such as V_D/V_T, PVR and compliance. It was concluded that platelet entrapment was a secondary phenomenon induced by previously damaged endothelium (Hechtman 1978a). Martin and coworkers (1981) studying hemorrhagic shock in dogs using a double indicator technique, demonstrated that pulmonary platelet uptake

was inversely related to pulmonary blood flow and could be reversed, i.e. washing platelets out of the lung by increasing blood flow. Thus it would appear that platelets play a secondary role in both oleic acid and endotoxin-induced lung injury.

Our determination of wet/dry ratio as an estimate of extravascular lung water demonstrated marked difference between the saline control animals and those given oleic acid. While no statistically significant difference occurred in the oleic acid groups, those receiving prostacyclin (3 and 5) tended to have a greater wet/dry ratio when compared to the nonprostacyclin groups (2 and 4). It is interesting to note that the prostacyclin groups had the lowest calculated residual lung blood volume suggesting an increase in extravascular water. There are two possible explanations. First is that of Prewitt and Wood (1979) who suggested that increasing pulmonary blood flow with a vasodilator will increase the amount of edema present by increasing the hydrostatic pressure in injured areas. Secondly, prostacyclin may potentiate swelling induced by other mediators (Murota and Murita 1978) due to vasodilation and increased blood flow to the injured area.

It would appear that platelet manipulation with prostacyclin and/or a phosphodiesterse inhibitor made no

difference to the degree of pulmonary edema. Perhaps treatment with prostacyclin or phosphodiesterase inhibitors prior to oleic acid injection would have produced a different result. However, this is clinically impractical. As mentioned earlier, it would have been beneficial to have measured bleeding time to confirm platelet aggregation inhibition.

Slotman et al (1982) that dogs receiving oleic acid followed by a prostacylin infusion demonstrated few pulmonary histological abnormalities at autopsy (Slotman et al, 1982). This is hard to rationalize. Oleic acid produces an extremely patchy distribution of pulmonary pathology with dependent lung regions having a greater proportion of damage. The results of our study indicate no difference in histology, wet/dry ratio or indium^{111} uptake between all the oleic acid groups whether or not prostacyclin was infused. Inspection of the oleic acid damaged lungs at autopsy revealed some normal areas particularly in the least dependent regions. Perhaps these were the areas biopsied in Slotman's study. Another possibility is that the prostacyclin dose of 300ng/kg/min was required to attain a pharmacological effect. The result from this study showing no trend towards improvement in the prostacyclin infused animals with respect to wet-dry ratio or indium^{111} uptake makes the latter possibility unlikely.

A curious finding occurred when residual pulmonary blood volume was examined. It appeared that the oleic acid injury brought about a reduction in residual pulmonary blood volume. This reduction was not significant when compared to the control group but the prostacyclin oleic acid groups (3 and 5) had a signifcantly lower residual blood volume than the nonprostacyclin oleic acid groups. As mentioned above, the prostacyclin oleic acid groups had a greater wet/dry ratio than the nonprostacyclin oleic acid groups (2 and 4) suggesting a greater amount of edema fluid. Perhaps increased interstitial fluid accumulation encroached upon and displaced blood from the pulmonary capillary bed. Certainly, the increased lung count rate in the oleic acid groups appears to be on the basis of platelet sequestration and not increased pulmonary capillary blood volume.

This study was beset by several problems which delayed its conclusion. First, the initial batch of prostacyclin was found to be impotent. When tested against human platelets, as described in the prostacyclin potency study as found in Appendix IIa, it was discovered that a dilution of 100ng/ml was required to inhibit human platelet aggregation induced by ADP. This batch of drug was not used. The problem was traced to a delay in

shipping and new drug was eventually obtained. The second problem occurred after the group 5 (oleic acid, prostacyclin and aminophylline) animals were studied. The refrigeration unit storing the prostacyclin was inadvertently turned off and the problem was not discovered for three days. The prostacyclin solution attained room temperature for an unknown period of time. When the remaining prostacyclin solution was assayed for potency against human platelets as previously described, 4ng/ml of this solution totally inhibited platelet aggregation. This suggested a small loss of potency; however, platelet studies of this nature are not very sensitive and have a large experimental error. A third problem encountered was the degree of variability in the extent of pulmonary injury induced by oleic acid. As the initial oleic acid in the lab was of indeterminant age, it was assayed and found to have only 47% oleic acid with a large number of impurities and breakdown products of unknown pharmacological effect. In order to make this study comparable to others a new supplier guaranteeing 99% oleic acid was obtained. This resulted in better reproducibility of pulmonary injury. The data from five dogs given detective oleic acid was rejected from the study because of the unpredictable results obtained. Therefore all dogs reported in the

study received 99% pure oleic acid. As well, one dog was examined as a control at the beginning of the experiment. The results from this trial run were also not included.

A final problem developed in attempting to measure end tidal carbon dioxide concentrations. The carbon dioxide analyzer used was difficult to calibrate and had an extremely large baseline drift. Because of the questionable reliability of these values, they have not been included in the results section.

Another possible criticism of this study was that bleeding time was not measured. Bleeding time can be used as a rough estimate of in vivo platelet function. Ubatuba and coworkers (1979) determined that bleeding time lengthened as the amount of prostacyclin infused increased to a maximum dose of 100ng/kg/min in rabbits. Further increases in the amount of prostacyclin infused after this point had no further effect. As well, the difference in bleeding time between the doses of 50 to 100ng/kg/min was small (Ubatuba et al, 1979). However, observation of bleeding time might have shed light on any differences found in group 6 (prostacyclin only) after the previously mentioned refrigeration problem. The effects of oleic acid administration on bleeding time are unknown. Perhaps this would have been a useful adjunct

in monitoring our animals and should be included in any further study.

Serum aminophylline levels were not obtained. While the aminophylline dose was individualized on a weight basis, it is possible that oleic acid administration could alter the volume of distribution of the drug leading to underdosing of the animal. Other extraneous factors such as age or health prior to the experiment could alter drug distribution or excretion resulting in a varying pharmacological effect. The assay available for theophylline had not been calibrated on dog blood and this cost would have proven to be prohibitive.

Finally, many of the results are difficult to interpret statistically because of wide variations within each group. Certainly, a larger number of test subjects in each group, such as fifteen or more may have presented a clearer trend. Despite all the above mentioned problems and criticisms, this study presents some interesting results from which valid conclusions may be drawn.

Thus, it would appear that the only beneficial effect of prostacyclin in oleic acid-induced pulmonary injury is that of a vasodilator increasing cardiac output, tissue perfusion and tissue substrate availability. Platelet aggregation inhibition plays a

minimal role and appears to be ineffective in this model. As well, it appears that platelets play, at best, a minimal role in oleic-acid induced pulmonary injury. Leukocytes and the leukotriene pathway are far more important mediators of lung injury (Wedmore and William, 1980). Obviously, further investigation along this avenue is required.

The role of platelets in oleic acid-induced pulmonary injury could be settled by administering antiplatelet serum to render test animals thrombocytopenic prior to oleic acid injection. Under these experimental conditions the study of hemodynamic and respiratory parameters as well as pulmonary histology and extravascular lung water would indicate whether platelets had a role to play in this model or were sequestered in the lung secondarily. Perhaps a small number of animals should be restudied with oleic acid and prostacyclin (group 3), this time adding bleeding time and serum theophylline levels to the other blood tests. Hopefully, this would ensure adequate drug levels for pharmacological effect. Finally, a study utilizating radiolabelled polymorphonuclear leukocytes in the endotoxin-induced pulmonary injury model should be undertaken to observe if prostcyclin infusion and subsequent modulation of leukocyte function is of any clinical significance.

Prostacyclin has already undergone several clinical trials as an anticoagulant in extracorporeal circulations. This drug has been used successfully with cardiopulmonary bypass, dialysis and charcoal hemoperfusion. Because of its potent vasodilator properties, prostacyclin has found limited clinical use in other areas. This drug is extremely expensive (approximately $300.00 U.S. per mg). Prostacyclin is very sensitive to both pH and temperature changes. This makes it very difficult to use in clinical situations. Prostacyclin at room temperature and pH 10 could loose 10% of its potency every 6 hours, an impractical situation for a continuous infusion. While vascular dilatation remains a potent effect of this drug, other potential benefits such as aggregation, inhibition or leukocyte modulation appear to have questionable effect in the oleic acid model. Other vasodilators such as sodium nitroprusside, hydralazine or phentolamine have similar pharmacological properties albeit through different mechanisms. However, these agents have advantages. In the present state of the art, prostacyclin has little practical effect as a therapeutic agent.

In summary, prostacyclin appears to act as a vasodilator in the oleic acid-induced pulmonary injury model. This agent effects a reduction in MAP and SVR while maintaining cardiac output at control values in the oleic acid model. The previously mentioned hemodynamic profile has been demonstrated by others using prostacyclin in this model (Slotman et al, 1982). A similar hemodynamic pattern occurred when sodium nitroprusside was used in the oleic acid model (Prewitt and Wood 1981). Prostacyclin appears to improve tissue oxygenation as evidenced by a greater $P\bar{v}O_2$ and $A\text{-}VDO_2$ when compared to the oleic acid controls. However, this did not reflect in an improved PaO_2 in those animals. In fact, $\dot{Q}_S/\dot{Q}_T$ rose in the prostacyclin animals presumably by increasing blood flow through a fixed shunt. Finally, neither prostacyclin or aminophylline altered pulmonary histology, wet/dry ratio or pulmonary platelet sequestration. These facts suggest that prostacyclin has no advantages over other vasodilators. Secondly, platelet sequestration appears to be a secondary phenomenon and platelet modulation with prostacyclin does not alter outcome. Because of the high cost and unstable nature of prostacyclin and better alternatives available as vasodilators, prostacyclin cannot be recommended for clinical use in hypoxemic respiratory failure at this point in time.

Summary of Conclusions

1) Prostacyclin exerts its beneficial effect by vasodilation thus improving cardiac output and tissue oxygen and substrate delivery.

2) Platelets appear to play a passive role in oleic acid induced pulmonary injury being sequestered after endothelial injury. Certainly platelet modulation by prostacyclin had no effect on pulmonary pathology, wet/dry ratio or pulmonary platelet sequestration.

3) Prostacyclin does not reduce pulmonary extravascular water accumulation in oleic acid-induced damage.

4) Platelet aggregation inhibition with prostacylin appears not to be useful in preventing or reversing oleic acid-induced lung damage.

5) Histological findings were unchanged with the use of prostacyclin.

6) Prostacyclin is too unstable and expensive to have much practical clinical use.

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APPENDIX I

To be discussed in this section are the composition and methods of production of various solutions used during the animal experiment.

a) Acid Citrate Dextrose Solution: the acid citrate dextrose solution (ACD) was prepared by a formula kindly supplied by Mr. R. Schmidt and Dr. Z. Catz of the Department of Nuclear Medicine at the W.W. Cross Cancer Institute. The solution was produced in batches and refrigerated for subsequent use. Ingredients included: dextrose 1.3gm, sodium citrate 2.5gms and citric acid 0.8gm. The foregoing were dissolved and mixed in distilled water and refrigerated until used.

b) ACD:NS Solution: 1ml of the previously described ACD solution was added to 7ml of sterile normal saline. The resulting mixture, was placed on a vortex mixer and 1 M sodium hydroxide was added to achieve a pH of 6.5. This solution underwent immediate use and was discarded at the end of each individual trial.

c) Tris Buffer: to prepare a 50mMol concentration, 0.788gm of hydroxymethylaminoethane (Tris buffer) was

added to 100ml of distilled water. This solution was then cooled to 4°C, as the pH of tris buffer is temperature dependent. The pH was adjusted to 11 by the careful titration of 1 M sodium hydroxide. This solution was prepared in batches and refrigerated for future use.

d) Prostacyclin: prostacyclin (PGI_2) was suppled as the sodium salt. The PGI_2 was weighed and added to 50mMol tris buffer pH 11, and cooled to 0°C to form a final concentration of 500µg/ml. The solution was then separated into 1ml aliquots and stored at -70°C. In this state, PGI_2 should have greater than 95% potency at 6 months. At time of utilization, the PGI_2 was warmed to 0°C in an ice water bath. The 500µg/ml solution was further diluted with tris buffer (pH 11) to produce a final concentration of 1200ng/kg/ml. Based on the weight of the animal to be studied, the drug was infused at 5ml/hr, of vehicle, by a Harvard infusion pump resulting in a final dose of 100ng/kg/min. This produced a constant volume of buffer infused into all animals. The prostacyclin syringe, was placed in a cooling jacket and continuously bathed with ice water (temperature 0°C) while the infusion took place.

e) Formalin Solution: ingredients for formalin included 1M sodium hydroxide 500ml, 3.8% sodium citrate 500ml and 10% formaldehyde. These constituents were then combined resulting in a 10% formalin solution.

f) Drabkin's Solution: the ferricyanide technique for hemoblobin determination was utilized. Ingredients for Drabkin's solution included potassium cyanide 0.5gms, potassium ferricyanide 0.2gms and distilled water 1000ml. These constituents were combined to make Drabkin's solution. Ferricyanide converts the iron in the hemoglobin molecule from the ferrous to the ferric state thus producing methemoglobin. Methemoglobin then combines with potassium cyanide to produce cyanomethemoglobin which has a peak optical density at 525μm. Both the Coulter hemoglobinometer and the Klett Sumerson colormeter measure light absorption at this position of the color spectrum. The Klett Sumerson colormeter was calibrated with standardized controls as well as dog blood submitted to itself and the Coulter hemoglobinometer.

APPENDIX II

a) Prostacyclin Potency Study:

Platelets were collected from human volunteers. Aggregation studies were then performed in the presence and absence of prostacyclin.

i) Blood Collection:

43ml of whole blood was collected in a 50ml syringe which already contained 7ml of 3.8% sodium citrate solution as an anticoagulant. The blood was withdrawn slowly via an antecubital vein through an 18 gauge needle to avoid platelet trauma. The blood was then centrifuged at 170g for 20 min to allow for the separation of the red cells from the platelet rich plasma (PRP). The PRP was drawn off and held at room temperature.

ii) Platelet Function Studies:

These trials were divided into two groups.

Group I, which were the controls, consisted of placing 0.5ml of the PRP obtained above in a Payton dual chamber aggregometer. The plasma was warmed to 37°C. Aggregation was induced by adding 1µl of a 10^{-3} M solution of adenosine diphosphate (ADP) to the plasma. This resulted in a final concentration 1×10^{-6} M of ADP.

Group II, prostacyclin, in various concentrations ranging from $2.7 - 10.7 \times 10^{-9}$ M, was incubated with the PRP at 37°C for one minute, prior to the addition of ADP.

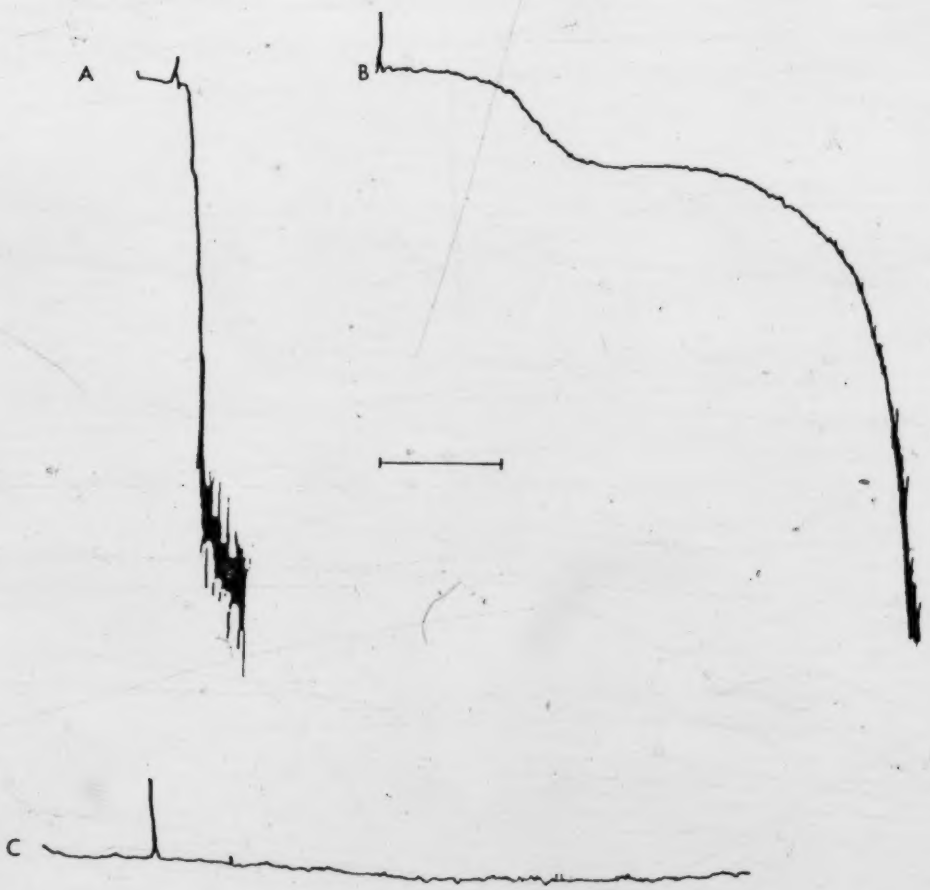
iii) Results:

Aggregation of platelets was indicated when a sudden profound decrease in optical density took place (Born 1962). In the control group, aggregation occurred 46 seconds after the addition of the ADP solution (Figure 12). In contrast, when plasma was incubated with prostacyclin at a concentration of 5.3×10^{-9} M a biphasic response at 2 minutes and 32 seconds duration occurred, indicating partial inhibition of aggregation. Finally, prostacyclin concentration of 8.0×10^{-9} M completely inhibited platelet aggregation. These results compare favorably with those obtained in other laboratories (Moncada et al, 1976; Ubatuba et al, 1979).

b) Platelet Survival Study: as a quality control project, platelet survival time after indium^{111}-oxine radiolabelling was investigated to determine the degree of damage inflicted on the platelets during the labelling process. As well, several other parameters were studied to determine the degree of efficiency and specificity of the labelling process.

Essentially platelets were collected and labelled as described in the radiolabelling portion of the material and methods section. The labelled platelet concentrate was reinfused through a forelimb view via an 18 gauge

Figure 12 - Platelet Function Curves
Optical Density on the Abcissa,
Time on the Ordinate
A: Control - note the marked increase in optical density with the addition of 1ul of 10^{-3}M ADP
B: Prostacyclin - 5.3×10^{-9}M produced a bephasic response
C: Prostacyclin - 8.0×10^{-9}M no response to ADP occurred



needle. Approximately 5ml of blood was collected from the opposite forelimb at 5 mins, 1, 24, 72, 92, 116, 140 and 164 hours after infusion of the radiolabelled platelets. One half of this sample was set aside for whole blood radioactivity determination, while the remaining half was fractionated in a centrifuge. Radioactivity was determined for the platelet rich plasma and packed cell fractions. The reason for counting the blood components independently was to determine whether the radiolabel remained platelet bound and to look for contamination of other cell types during the labelling process.

The amount of radiolabelled platelets remaining in the intravascular compartment was calculated and expressed as a fraction of the total count infusion from the following equation:

$$\text{Fraction of label remaining in the intravascular compartment} = \frac{Cpm_{WB} \times 75 \times wt}{Cpm_I \times Vol}$$

where: Cpm_{WB} represents radioactivity of 1ml whole blood

Wt represents animal weight in kg

Cpm_I represents count rate of a representative sample of radiolabelled platelet concentrate

and Vol represents volume of radiolabelled concentrate.

Results

The results are presented in Table 6. Generally only a small amount of radioactivity appeared in the packed cell fraction. The explanation probably lies in the fact that some platelets get trapped in the packed cell fraction. Regression analysis of the decay of radioactivity in the intravascular volume revealed a straightline with the following equation:

$$y = 36.7 - 0.185x.$$

This resulted in a survival time of 189 hours, a result which compares favorably to those obtained by others (Thakur et al, 1976; Goodwin et al, 1978 and Lotter et al, 1980). While 30-40% of the labelled platelets could be recovered in the circulating blood pool one hour after infusion, the remainder were sequestered by the liver and spleen as confirmed by external gamma scintigraphy (Lotter et al, 1980). Undoubtedly, some platelets are damaged during the radiolabelling process; however, labelling with the indium^{111}-oxine results in a large number of functional labelled platelets.

c) Indium Oxine Quality Testing: in order to determine whether indium^{111} remained complexed and had not disassociated from the oximen moiety, 26 μCi were added to a test tube containing equal parts of chloroform and

Table 6 - Platelet Survival Study

		Radioactivity -background CPM		Radioactivity 1u/ml CMP	% calculated fraction of labelled platelets remaining in the intravascular space
5min	WB		1490	1490	34.3%
	PRP	4025	2013	-	
	PC		365	365	-
1hr	WB	1931	1931	44.5%	
	PRP	3499	1750	-	
	PC		213	213	-
24hr	WB	1317	1317	30.4%	
	PRP	4508	2147	-	
	PC		233	233	-
72hr	WB	1617	1617	37.3%	
	PRP	2165	1274	-	
	PC		110	110	-
92hr	WB	883	883	20.4%	
	PRP	1618	852	-	
	PC		112	112	-
116hr	WB	439	439	10.1%	
	PRP	805	592	-	
	PC		112	112	-
140hr	WB	277	277	6.4%	
	PRP	396	229	-	
	PC		77	77	-
164hr	WB	94	94	2.2%	
	PRP	151	116	-	
	PC		26	26	-

Standard from platelet concentrate 35417cpm in 25µl of solution.

water. This mixture was then vigorously agitated for 20 minutes and set aside to allow the water and chloroform phases to fully separate. The water was decanted off the chloroform and each were placed in a dose calibrator to determine radioactivity. All radioactivity remained in the chloroform phase while none was found in the water phase. It was concluded that all the indium^{111} remained complexed to the oxine moiety, therefore all the indium^{111} was available for cellular labelling.

d) Platelet Labelling Efficiency: to determine the uptake of radiolabel, 5ml of platelet concentration (715,000 platelets/ml) were incubated with 271μCi of indium^{111}-oxine as described in the radiolabelling portion of the material and methods section. After an incubation of 20 minutes, 1ml of PPP was added and the resulting mixture was placed in the centrifuge and spun at 1400g for 14 minutes. The resulting supernatant was set aside for radioactivity quantitation. The platelets were washed with 4ml of PPP and again centrifuged at 1400g for 14 minutes. This wash solution was also set aside for quantitation of radioactivity. The platelets were resuspended in 5ml of PPP. The platelet suspension, supernatant and wash solution were all placed in a dose calibrator. The results indicated that 232μCi were found

in the platelet concentrate while 36 and 3 μCi were found in the supernatant and wash solution respectively. This yielded a labelling efficiency of 85.6%. Again, this compares favorably with previous studies.

e) Purity of Platelet Concentrate: the platelet concentrate was examined prior to addition of radiolabel to determine the number of contaminating white and red blood cells. A sample of platelet concentrate was hand counted in a counting chamber, as the numbers of cells involved could conceivably be below the sensitivity of automatic counting equipment. No red blood cells were observed. Only one leukocyte was seen for every 100,000 platelets, that is an average of 4 leukocytes per cumm. This is a very small contamination of platelet concentrate and should not lead to any significant error. As well, no platelet aggregation was seen with light microscopy.

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